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【書類名】明細書
【発明の名称】アンチセンス核酸
【技術分野】
【０００１】
本発明は、ヒトジストロフィン遺伝子の第53番目のエクソンのスキッピングを可能にするアンチセンスオリゴマー及び該オリゴマーを含む医薬組成物に関する。
【背景技術】
【０００２】
デュシェンヌ型筋ジストロフィー（DMD）は出生男子約3,500人に1人が発症する最も頻度の高い遺伝性進行性筋萎縮症である。乳幼児期には正常のヒトとほとんど変わらない運動機能を示すが、4～5歳頃から筋力低下がみられる。その後筋力低下は進行し12歳頃までに歩行不能になり、20歳代で心不全または呼吸器不全により死に至る重篤な疾患である。現在、DMDに対する有効な治療法はなく、新たな治療薬の開発が強く求められている。
【０００３】
DMDはジストロフィン遺伝子の変異が原因であることが知られている。ジストロフィン遺伝子はX染色体に存在し、220万塩基のDNAから成る巨大な遺伝子である。DNAからmRNA前駆体に転写され、さらにスプライシングによりイントロンが除かれ79のエクソンが結合したmRNAは11,058塩基になる。このmRNAから3,685のアミノ酸に翻訳され、ジストロフィンタンパク質が生成される。ジストロフィンタンパク質は筋細胞の膜安定性の維持に関与しており、筋細胞を壊れにくくするために必要である。DMD患者のジストロフィン遺伝子は変異を有するため、筋細胞において機能を持つジストロフィンタンパク質が殆ど発現されない。そのため、DMD患者体内では、筋細胞の構造を維持できなくなり、多量のカルシウムイオンが筋細胞内に流れ込む。その結果、炎症に似た反応が生じ、線維化が進むために筋細胞が再生されにくくなる。
【０００４】
ベッカー型筋ジストロフィー（BMD）もジストロフィン遺伝子の変異が原因であるが、その症状は筋萎縮による筋力低下を呈するものの一般にDMDと比較して軽く、筋力低下の進行も遅く、多くの場合、成人期に発症する。DMDとBMDとの臨床症状の違いは、変異によりジストロフィンmRNAがタンパク質へと翻訳される際のアミノ酸読み取り枠が破壊されるか、あるいは維持されるかによるものと考えられている（非特許文献1）。つまり、DMDでは、アミノ酸読み取り枠がずれる変異を有することにより、機能を持つジストロフィンがほとんど発現しないが、BMDでは変異によりエクソンの一部は欠失しているが、アミノ酸読み取り枠は維持されているために不完全ながらも機能を有するジストロフィンタンパク質が産生される。
【０００５】
DMD の治療法として、エクソンスキッピング法が期待されている。この方法は、スプライシングを改変することでジストロフィンのmRNAのアミノ酸読み取り枠を修復し、部分的に機能を回復したジストロフィンの発現を誘導する方法である（非特許文献2）。変異及びエクソンスキッピングの対象となるアミノ酸配列部分は失われることになる。そのためこの治療で発現されるジストロフィンタンパク質は正常のものより短くなるが、アミノ酸読み取り枠が維持されるために筋細胞を安定化する機能が部分的に保持される。従って、エクソンスキッピングにより、DMDは、より軽症のBMDと同じような症状を呈するようになると期待されている。エクソンスキッピング法は、マウスやイヌによる動物実験を経て、ヒトDMD患者に対する臨床試験が行われている。
【０００６】
エクソンスキッピングは、5’又は3’スプライス部位のいずれか若しくは両方、又はエクソンの内部を標的とするアンチセンス核酸の結合により誘導することができる。エクソンは両方のスプライス部位がスプライソソーム複合体によって認識された場合のみmRNAに包含される。従って、スプライス部位をアンチセンス核酸でターゲッティングすることにより、エクソンスキッピングを誘導することができる。また、エクソンがスプライシング

の機構に認識されるためにはエクソンスプライシングエンハンサー（ESE）へのSRタンパク質の結合が必要であると考えられており、ESEをターゲッティングすることでもエクソンのスキッピングを誘導することができる。

【０００７】

ジストロフィン遺伝子の変異はDMD患者によって異なるため、遺伝子変異の場所や種類に応じたアンチセンス核酸が必要になる。これまでに、西オーストラリア大学のSteve Wiltonらによって79個全てのエクソンに対してエクソンスキッピングを誘導するアンチセンス核酸が作製されており（非特許文献3）、オランダのAnnemieke Aartsma-Rusらによって39種類のエクソンに対してエクソンスキッピングを誘導するアンチセンス核酸が作られている（非特許文献4）。

【０００８】

全DMD患者の8%程度は、第53番目のエクソン（以下、「エクソン53」という）をスキッピングすることで治療可能と考えられている。近年では、ジストロフィン遺伝子のエクソン53をエクソンスキッピングのターゲットとした研究について、複数の研究機関から報告がなされている（特許文献1～3；非特許文献5）。しかしながら、エクソン53を高効率にスキッピングさせる技術は、いまだに確立されていない。

【先行技術文献】

【特許文献】

【０００９】

【特許文献１】国際公開公報 WO 2006/000057
【特許文献２】国際公開公報 WO 2004/048570
【特許文献３】米国特許公開公報 US 2010/0168212

【非特許文献】

【００１０】

【非特許文献１】Monaco A. P. et al., Genomics 1988; 2: p. 90-95
【非特許文献２】Matsuo M., Brain Dev 1996; 18: p. 167-172
【非特許文献３】Wilton S. D., et al., Molecular Therapy 2007: 15: p. 1288-96
【非特許文献４】Annemieke Aartsma-Rus et al., (2002) Neuromuscular Disorders 12: S71-S77
【非特許文献５】Linda J. Popplewell et al., (2010) Neuromuscular Disorders , vol. 20, no. 2, p. 102-10

【発明の概要】

【発明が解決しようとする課題】

【００１１】

上記のような状況において、ジストロフィン遺伝子のエクソン53のスキッピングを強く誘導するアンチセンスオリゴマー及びそのオリゴマーを含む筋ジストロフィー治療薬が望まれている。

【課題を解決するための手段】

【００１２】

本発明者らは、エクソン53に変異を有するジストロフィン変異遺伝子の構造を詳細に研究した結果、ジストロフィン遺伝子のmRNA前駆体（以下、「pre-mRNA」という）のうちエクソン53の第32～56番目周辺のヌクレオチドからなる塩基配列をアンチセンスオリゴマーでターゲッティングすることにより、高効率にエクソン53のスキッピングを誘導できることを見出した。本発明者らは、この知見に基づき、本発明を完成させた。

【００１３】

即ち、本発明は、以下のとおりである。

[1] ヒトジストロフィン遺伝子の第53番目のエクソンのスキッピングを可能にするアンチセンスオリゴマーであって、ヒトジストロフィン遺伝子の第53番エクソンの5’末端から第31～53番目、第31～54番目、第31～55番目、第31～56番目、第31～57番目、第31～58

番目、第32～53番目、第32～54番目、第32～55番目、第32～56番目、第32～57番目、第32～58番目、第33～53番目、第33～54番目、第33～55番目、第33～56番目、第33～57番目、第33～58番目、第34～53番目、第34～54番目、第34～55番目、第34～56番目、第34～57番目、第34～58番目、第35～53番目、第35～54番目、第35～55番目、第35～56番目、第35～57番目、第35～58番目、第36～53番目、第36～54番目、第36～55番目、第36～56番目、第36～57番目又は第36～58番目のヌクレオチドからなる配列のいずれか1つに相補的な塩基配列からなる、アンチセンスオリゴマー。

[2] オリゴヌクレオチドである、前記[1]に記載のアンチセンスオリゴマー。

[3] 前記オリゴヌクレオチドを構成する糖及び/又はリン酸の少なくとも１つが修飾されている、前記[2]に記載のアンチセンスオリゴマー。

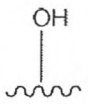
[4] 前記オリゴヌクレオチドを構成する糖の2'位の水酸基が、OR、R、R'OR、SH、SR、NH_2、NHR、NR_2、N_3、CN、F、Cl、Br及びIからなる群より選択されるいずれかの基で置換された、前記[3]に記載のアンチセンスオリゴマー。（上記Rは、炭素数1～6のアルキル又は炭素数1～6のアリールを示し、上記R'は、炭素数1～6のアルキレンを示す。）

[5] 前記オリゴヌクレオチドを構成するリン酸が、ホスホロチオエート、ホスホロジチオエート、アルキルホスホネート及びホスホロアミデートからなる群より選択されるいずれか1つのものである、前記[3]又は[4]に記載のアンチセンスオリゴマー。

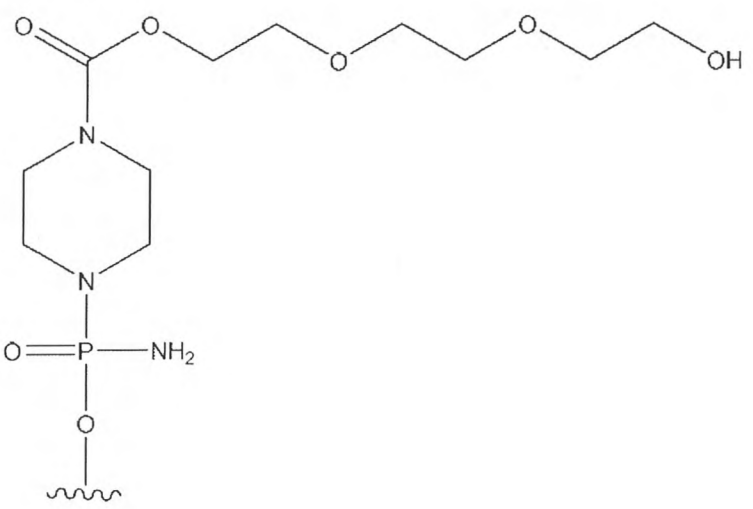
[6] モルホリノオリゴマーである、前記[1]に記載のアンチセンスオリゴマー。[7] ホスホロジアミデートモルホリノオリゴマーである、前記[6]に記載のアンチセンスオリゴマー。

[8] 5'末端核酸残基のリボース又はモルホリノ環に結合した5'メチレンが、下記のいずれかの基により修飾されている、前記[1]～[7]のいずれか1項に記載のアンチセンスオリゴマー。

【化１】

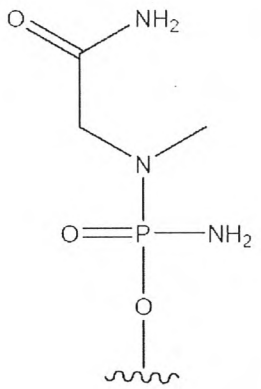


【化２】



、又は

【化３】



[9] ヒトジストロフィン遺伝子の第53番エクソンの5’末端から第32～56番目又は第36～56番目のヌクレオチドからなる配列に相補的な塩基配列からなる、前記[1]～[8]のいずれか1項に記載のアンチセンスオリゴマー。
[10] 配列番号2～37からなる群より選択されるいずれか1つの塩基配列からなる、前記[1]～[8]のいずれか1項に記載のアンチセンスオリゴマー。
[11] 配列番号11、17、23、29及び35からなる群より選択されるいずれか1つに示す塩基配列からなる、前記[1]～[8]のいずれか1項に記載のアンチセンスオリゴマー。
[12] 配列番号11又は35に示す塩基配列からなる、前記[1]～[8]のいずれか1項に記載のアンチセンスオリゴマー。
[13] 前記[1]～[12]のいずれか1項に記載のアンチセンスオリゴマー、その医薬的に許容可能な塩又は水和物を有効成分とする、筋ジストロフィー治療用医薬組成物。
【発明の効果】
【００１４】
本発明のアンチセンスオリゴマーにより、ジストロフィン遺伝子のエクソン53のスキッピングを高効率に誘導することが可能である。また、本発明の医薬組成物を投与することにより、デュシェンヌ型筋ジストロフィーの症状を、効果的に軽減することができる。
【図面の簡単な説明】
【００１５】
【図１】ヒト横紋筋肉腫細胞株（RD細胞）におけるジストロフィン遺伝子エクソン53のスキッピング効率を示す図である。
【図２】ヒト正常組織由来線維芽細胞（TIG-119細胞）にヒトmyoD遺伝子を導入して筋肉細胞に分化誘導した細胞におけるジストロフィン遺伝子のエクソン53のスキッピング効率を示す図である。
【図３】ヒトDMD患者由来線維芽細胞（5017細胞）にヒトmyoD遺伝子を導入して筋肉細胞に分化誘導した細胞におけるジストロフィン遺伝子のエクソン53のスキッピング効率を示す図である。
【発明を実施するための形態】
【００１６】
1．アンチセンスオリゴマー
本発明は、ヒトジストロフィン遺伝子の第53番目のエクソンのスキッピングを可能にするアンチセンスオリゴマーであって、ヒトジストロフィン遺伝子の第53番エクソンの5’末端から第31～53番目、第31～54番目、第31～55番目、第31～56番目、第31～57番目、第31～58番目、第32～53番目、第32～54番目、第32～55番目、第32～56番目、第32～57番目、第32～58番目、第33～53番目、第33～54番目、第33～55番目、第33～56番目、第33～57番目、第33～58番目、第34～53番目、第34～54番目、第34～55番目、第34～56番目、第34～57番目、第34～58番目、第35～53番目、第35～54番目、第35～55番目、第35～56番目、

第35～57番目、第35～58番目、第36～53番目、第36～54番目、第36～55番目、第36～56番目、第36～57番目又は第36～58番目のヌクレオチドからなる配列のいずれか1つに相補的な塩基配列からなる、アンチセンスオリゴマー（以下、「本発明のオリゴマー」という）、を提供する。

【００１７】

[ヒトジストロフィン遺伝子の第53番目のエクソン]

本発明において、「遺伝子」には、ゲノム遺伝子以外に、cDNA、mRNA前駆体及びmRNAも含まれる。好ましくは、遺伝子は、mRNA前駆体、即ち、pre-mRNAである。

ヒトゲノムにおいて、ジストロフィン遺伝子は遺伝子座Xp21.2に存在する。ジストロフィン遺伝子は、3.0 Mbpのサイズを有しており、既知のヒト遺伝子としては最大の遺伝子である。但し、ジストロフィン遺伝子のコード領域はわずか14kbに過ぎず、該コード領域は79個のエクソンとしてジストロフィン遺伝子内に分散している（Roberts, RG., et al., Genomics, 16: 536-538 (1993)）。ジストロフィン遺伝子の転写物であるpre-mRNAは、スプライシングを受けて14kbの成熟mRNAを生成する。ヒトの野生型ジストロフィン遺伝子の塩基配列は公知である（GenBank Accession No. NM_004006）。

ヒトの野生型ジストロフィン遺伝子のエクソン53の塩基配列を配列番号1に示す。

【００１８】

本発明のオリゴマーは、エクソン53のスキッピングにより、DMD型ジストロフィン遺伝子にコードされるタンパク質を、BMD型ジストロフィンに改変することを目的として作製されたものである。従って、本発明のオリゴマーのエクソンスキッピングの対象となるジストロフィン遺伝子のエクソン53には、野生型だけではなく、変異型も含まれる。

変異型のヒトジストロフィン遺伝子のエクソン53は、具体的には、以下の（a）又は（b）に記載のポリヌクレオチドである。

（a）配列番号1の塩基配列と相補的な塩基配列からなるポリヌクレオチドとストリンジェントな条件下でハイブリダイズするポリヌクレオチド；及び

（b）配列番号1の塩基配列に対して、90％以上の同一性を有する塩基配列からなるポリヌクレオチド

【００１９】

本明細書中、「ポリヌクレオチド」とは、DNA又はRNAを意味するが、好ましくは、RNAである。

本明細書中、「ストリンジェントな条件下でハイブリダイズするポリヌクレオチド」とは、例えば、配列番号1の塩基配列と相補的な塩基配列からなるポリヌクレオチドの全部又は一部をプローブとして、コロニーハイブリダイゼーション法、プラークハイブリダイゼーション法又はサザンハイブリダイゼーション法などを用いることにより得られるポリヌクレオチドをいう。ハイブリダイゼーションの方法としては、例えば、"Sambrook & Russell, Molecular Cloning: A Laboratory Manual Vol. 3, Cold Spring Harbor, Laboratory Press 2001"及び"Ausubel, Current Protocols in Molecular Biology, John Wiley & Sons 1987-1997"などに記載されている方法を利用することができる。

【００２０】

本明細書中、「ストリンジェントな条件」とは、低ストリンジェントな条件、中ストリンジェントな条件及び高ストリンジェントな条件のいずれでもよい。「低ストリンジェントな条件」は、例えば、5×SSC、5×デンハルト溶液、0.5％SDS、50％ホルムアミド、32℃の条件である。また、「中ストリンジェントな条件」は、例えば、5×SSC、5×デンハルト溶液、0.5％SDS、50％ホルムアミド、42℃又は5×SSC、1％ SDS、50 mM Tris－HCl（pH7.5）、50％ホルムアミド、42℃の条件である。「高ストリンジェントな条件」は、例えば、5×SSC、5×デンハルト溶液、0.5％SDS、50％ホルムアミド、50℃又は0.2×SSC、0.1％ SDS、65℃の条件である。これらの条件において、温度を上げるほど高い同一性を有するポリヌクレオチドが効率的に得られることが期待できる。ただし、ハイブリダイゼーションのストリンジェンシーに影響する要素としては温度、プローブ濃度、プローブの長さ、イオン強度、時間、塩濃度等の複数の要素が考えられ、当業者であればこれらの要素

を適宜選択することで同様のストリンジェンシーを実現することが可能である。

【００２１】

なお、ハイブリダイゼーションに市販のキットを用いる場合は、例えばAlkphos Direct Labelling and Detection System (GE Healthcare) を用いることができる。この場合は、キットに添付のプロトコルにしたがい、標識したプローブとのインキュベーションを一晩行った後、メンブレンを55℃の条件下で0.1% (w/v) SDSを含む1次洗浄バッファーで洗浄後、ハイブリダイズしたポリヌクレオチドを検出することができる。あるいは、配列番号1の塩基配列と相補的な塩基配列の全部又は一部に基づいてプローブを作製する際に、市販の試薬（例えば、PCRラベリングミックス（ロシュ・ダイアグノス社）等）を用いて該プローブをジゴキシゲニン（DIG）ラベルした場合には、DIG核酸検出キット（ロシュ・ダイアグノス社）を用いてハイブリダイゼーションを検出することができる。

【００２２】

上記以外にハイブリダイズ可能なポリヌクレオチドとしては、相同性検索ソフトウェアであるBLASTにより、デフォルトのパラメーターを用いて計算したときに、配列番号1のポリヌクレオチドと90%以上、91%以上、92%以上、93%以上、94%以上、95%以上、96%以上、97%以上、98%以上、99%以上、99.1%以上、99.2%以上、99.3%以上、99.4%以上、99.5%以上、99.6%以上、99.7%以上、99.8%以上、又は99.9%以上の同一性を有するポリヌクレオチドをあげることができる。

【００２３】

なお、塩基配列の同一性は、カーリン及びアルチュールによるアルゴリズムBLAST (Basic Local Alignment Search Tool) (Proc. Natl. Acad. Sci. USA 872264-2268, 1990; Proc Natl Acad Sci USA 90: 5873, 1993) を用いて決定できる。BLASTのアルゴリズムに基づいたBLASTNやBLASTXと呼ばれるプログラムが開発されている (Altschul SF, et al: J Mol Biol 215: 403, 1990) 。BLASTNを用いて塩基配列を解析する場合は、パラメーターは、例えばscore = 100、wordlength = 12とする。BLASTとGapped BLASTプログラムを用いる場合は、各プログラムのデフォルトパラメーターを用いる。

【００２４】

エクソン53の5’末端から第31～53番目、第31～54番目、第31～55番目、第31～56番目、第31～57番目、第31～58番目、第32～53番目、第32～54番目、第32～55番目、第32～56番目、第32～57番目、第32～58番目、第33～53番目、第33～54番目、第33～55番目、第33～56番目、第33～57番目、第33～58番目、第34～53番目、第34～54番目、第34～55番目、第34～56番目、第34～57番目、第34～58番目、第35～53番目、第35～54番目、第35～55番目、第35～56番目、第35～57番目、第35～58番目、第36～53番目、第36～54番目、第36～55番目、第36～56番目、第36～57番目及び第36～58番目のヌクレオチドからなる配列に相補的な塩基配列の例を以下の表に示す。

【表１】

エクソン 53 のヌクレオチド	標的的配列	配列番号
第 31～53 番目	5’-CCGGTTCTGAAGGTGTTCTTGTA-3’	配列番号 2
第 31～54 番目	5’-TCCGGTTCTGAAGGTGTTCTTGTA-3’	配列番号 3
第 31～55 番目	5’-CTCCGGTTCTGAAGGTGTTCTTGTA-3’	配列番号 4
第 31～56 番目	5’-CCTCCGGTTCTGAAGGTGTTCTTGTA-3’	配列番号 5
第 31～57 番目	5’-GCCTCCGGTTCTGAAGGTGTTCTTGTA-3’	配列番号 6
第 31～58 番目	5’-TGCCTCCGGTTCTGAAGGTGTTCTTGTA-3’	配列番号 7
第 32～53 番目	5’-CCGGTTCTGAAGGTGTTCTTGT-3’	配列番号 8
第 32～54 番目	5’-TCCGGTTCTGAAGGTGTTCTTGT-3’	配列番号 9
第 32～55 番目	5’-CTCCGGTTCTGAAGGTGTTCTTGT-3’	配列番号 10
第 32～56 番目	5’-CCTCCGGTTCTGAAGGTGTTCTTGT-3’	配列番号 11
第 32～57 番目	5’-GCCTCCGGTTCTGAAGGTGTTCTTGT-3’	配列番号 12
第 32～58 番目	5’-TGCCTCCGGTTCTGAAGGTGTTCTTGT-3’	配列番号 13
第 33～53 番目	5’-CCGGTTCTGAAGGTGTTCTTG-3’	配列番号 14
第 33～54 番目	5’-TCCGGTTCTGAAGGTGTTCTTG-3’	配列番号 15
第 33～55 番目	5’-CTCCGGTTCTGAAGGTGTTCTTG-3’	配列番号 16
第 33～56 番目	5’-CCTCCGGTTCTGAAGGTGTTCTTG-3’	配列番号 17
第 33～57 番目	5’-GCCTCCGGTTCTGAAGGTGTTCTTG-3’	配列番号 18
第 33～58 番目	5’-TGCCTCCGGTTCTGAAGGTGTTCTTG-3’	配列番号 19
第 34～53 番目	5’-CCGGTTCTGAAGGTGTTCTT-3’	配列番号 20
第 34～54 番目	5’-TCCGGTTCTGAAGGTGTTCTT-3’	配列番号 21
第 34～55 番目	5’-CTCCGGTTCTGAAGGTGTTCTT-3’	配列番号 22
第 34～56 番目	5’-CCTCCGGTTCTGAAGGTGTTCTT-3’	配列番号 23
第 34～57 番目	5’-GCCTCCGGTTCTGAAGGTGTTCTT-3’	配列番号 24
第 34～58 番目	5’-TGCCTCCGGTTCTGAAGGTGTTCTT-3’	配列番号 25
第 35～53 番目	5’-CCGGTTCTGAAGGTGTTCT-3’	配列番号 26
第 35～54 番目	5’-TCCGGTTCTGAAGGTGTTCT-3’	配列番号 27
第 35～55 番目	5’-CTCCGGTTCTGAAGGTGTTCT-3’	配列番号 28
第 35～56 番目	5’-CCTCCGGTTCTGAAGGTGTTCT-3’	配列番号 29
第 35～57 番目	5’-GCCTCCGGTTCTGAAGGTGTTCT-3’	配列番号 30
第 35～58 番目	5’-TGCCTCCGGTTCTGAAGGTGTTCT-3’	配列番号 31
第 36～53 番目	5’-CCGGTTCTGAAGGTGTTC-3’	配列番号 32
第 36～54 番目	5’-TCCGGTTCTGAAGGTGTTC-3’	配列番号 33
第 36～55 番目	5’-CTCCGGTTCTGAAGGTGTTC-3’	配列番号 34
第 36～56 番目	5’-CCTCCGGTTCTGAAGGTGTTC-3’	配列番号 35
第 36～57 番目	5’-GCCTCCGGTTCTGAAGGTGTTC-3’	配列番号 36
第 36～58 番目	5’-TGCCTCCGGTTCTGAAGGTGTTC-3’	配列番号 37

【００２５】
本発明のオリゴマーは、好ましくは、ヒトジストロフィン遺伝子の第53番エクソンの5’末端から第32～56番目(配列番号11)、第33～56番目(配列番号17)、第34～56番目（配列番号23）、第35～56番目（配列番号29）又は第36～56番目（配列番号35）のヌクレオチドからなる配列のいずれか1つに相補的な塩基配列からなる。
好ましくは、本発明のオリゴマーは、ヒトジストロフィン遺伝子の第32～56番目(配列番号11)又は第36～56番目（配列番号35）のヌクレオチドからなる配列のいずれか1つに相補的な塩基配列からなるものである。
【００２６】
「ヒトジストロフィン遺伝子の第53番目のエクソンのスキッピングを可能にする」とは、ヒトジストロフィン遺伝子の転写物（例えば、pre-mRNA）のエクソン53に相当する部位に本発明のオリゴマーが結合することにより、該転写物がスプライシングを受けた際に、例えばエクソン52が欠失したDMD患者の場合、エクソン51の3’末端に相当する塩基配列の3’側にエクソン54の5’末端に相当する塩基配列が連結しており、コドンのフレームシフトが起こっていない成熟mRNAが形成されることを意味する。
従って、本発明のオリゴマーは、ヒトジストロフィン遺伝子のエクソン53のスキッピングを可能にする限り、ターゲット配列に対して100％相補的な塩基配列を有していなくてもよい。例えば、本発明のオリゴマーには、ターゲット配列に対して、1～3個、1～2個又は1個の非相補的塩基が含まれていてもよい。
【００２７】
ここで、前記「結合」は、本発明のオリゴマーとヒトジストロフィン遺伝子の転写物とを混合した場合に、生理的条件下で両者がハイブリダイズして二本鎖を形成することを意味する。上記「生理的条件下」とは、生体内と類似のpH、塩組成、温度に調節された条件を意味する。例えば、25～40℃、好ましくは37℃、pH 5～8、好ましくは、pH 7.4であって、塩化ナトリウム濃度が150 mMの条件が挙げられる。
【００２８】
ヒトジストロフィン遺伝子のエクソン53のスキッピングが生じたか否かは、ジストロフィン発現細胞（例えば、ヒト横紋筋肉腫細胞）に本発明のオリゴマーを導入し、前記ジストロフィン発現細胞のtotal RNAから、ジストロフィン遺伝子のmRNAのエクソン53の周辺領域をRT-PCR増幅し、該PCR増幅産物に対してnested PCR又はシークエンス解析を行うことにより確認することができる。　スキッピング効率は、ジストロフィン遺伝子のmRNAを被検細胞から回収し、該mRNAのうち、エクソン53がスキップしたバンドのポリヌクレオチド量「A」と、エクソン53がスキップしなかったバンドのポリヌクレオチド量「B」を測定し、これら「A」及び「B」の測定値に基づき、以下の式に従って計算することができる。

スキッピング効率（％）＝ A ／（ A ＋ B ）x 100

【００２９】
本発明のオリゴマーは、核酸のモノマーがリン酸エステル結合により連結したものであり、18～28 塩基の長さを有し、ヌクレオチドをモノマーとするオリゴマー、即ち、オリゴヌクレオチド（以下、「本発明のオリゴヌクレオチド」という）を含む。かかるヌクレオチドは、リボヌクレオチド又はデオキシリボヌクレオチドのいずれであってもよく、好ましくはリボヌクレオチドである。本発明のオリゴヌクレオチドは、各種自動合成装置（例えば、AKTA oligopilot plus 10 / 100（GE Healthcare））を用いて容易に合成することが可能であり、あるいは、第三者機関（例えば、Promega社又はTakara社）等に委託して作製することもできる。
【００３０】
また、本発明のオリゴヌクレオチドには、例えば、ヌクレアーゼ耐性等、生体内における安定性を高めるために、そのヌクレオチドを構成するリボース又はリン酸バックボーン

等の、少なくとも１つが修飾されているものも含まれる。かかる修飾としては、例えば、リボースの2’位の修飾、リボースのその他の部分の修飾、リン酸バックボーンの修飾を挙げることができる。リボースの2’位の修飾としては、例えば、リボースの2’位の水酸基をOR、R、R’OR、SH、SR、NH_2、NHR、NR_2、N_3、CN、F、Cl、Br、Iに置換する修飾を挙げることができる。ここで、Rはアルキル又はアリールを表す。Rのアルキルとしては、直鎖状または分枝鎖状の炭素数1～6のアルキルが好ましい。具体的には、例えば、メチル、エチル、n-プロピル、イソプロピル、n-ブチル、イソブチル、sec-ブチル、tert-ブチル、n-ペンチル、イソペンチル、ネオペンチル、tert-ペンチル、n-ヘキシル、およびイソヘキシルが挙げられる。当該アルキルは置換されていてもよく、かかる置換基としては、例えば、ハロゲン、アルキル、アルコキシ、シアノ、ニトロを挙げることができ、これらが1～3個置換されていてもよい。かかるハロゲンとしては、フッ素、塩素、臭素、ヨウ素を挙げることができる。アルキルとしては上記のアルキルを挙げることができる。アルコキシとしては直鎖状または分枝鎖状の炭素数1～6のアルコキシ、例えばメトキシ、エトキシ、n-プロポキシ、イソプロポキシ、n-ブトキシ、イソブトキシ、sec-ブトキシ、tert-ブトキシ、n-ペンチルオキシ、イソペンチルオキシ、n-ヘキシルオキシ、イソヘキシルオキシ等を挙げることができる。とりわけ、炭素数1～3のアルコキシが好ましい。Rのアリールとしては、炭素数6～10のアリールが好ましい。具体的には、例えば、フェニル、α-ナフチル、β-ナフチルを挙げることができる。とりわけフェニルが好ましい。また、R’はアルキレンを表す。R’のアルキレンとしては、直鎖状または分枝鎖状の炭素数1～6のアルキレンが好ましい。具体的には、例えば、メチレン、エチレン、トリメチレン、テトラメチレン、ペンタメチレン、ヘキサメチレン、2-（エチル）トリメチレン、1-（メチル）テトラメチレンを挙げることができる。リボースのその他の部分の修飾としては、例えば、4’位のOをSに置換する修飾を挙げることができる。また、本発明のオリゴヌクレオチドは、糖の 2’ 位と 4’ 位を- O - CH_2- で架橋することによりコンフォメーションを固定した人工核酸で構成されていてもよい。このような人工核酸としては、例えば、LNA（Locked Nucleic Acid）又はENA(2’-O,4’-C-Ethylene-bridged Nucleic Acids)などが挙げられるが、これらに限定されるものではない。

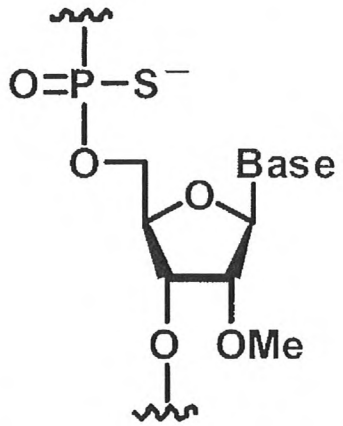
【００３１】

リン酸バックボーンの修飾としては、例えば、ホスホジエステル結合をホスホロチオエート結合、ホスホロジチオエート結合、アルキルホスホネート結合、ホスホロアミデート結合、ボラノフォスフェート結合（Enya et al: Bioorganic & Medicinal Chemistry , 2008, 18, 9154-9160 ）に置換する修飾を挙げることができる（例えば、特許再公表公報第2006/129594号及び第2006/038608号を参照）。

【００３２】

本発明のオリゴヌクレオチドは、好ましくは、リボースの2’位の水酸基が-OMe（Ｍｅ：メチル） で置換され、リン酸基の-O-が、-S-で置換された、2’-OMe-S-RNA（下記式参照）をモノマーとするオリゴマーである。

【化４】



（式中、Meは、メチルを示し、Baseは、アデニン、グアニン、ヒポキサンチン、シトシン、チミン又はウラシルのいずれかの塩基若しくは修飾塩基を示す。）

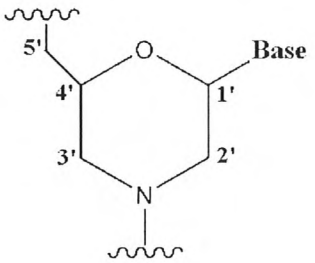
【００３３】

あるいは、本発明のオリゴマーは、ヌクレオチド類似体をモノマーとするオリゴマーであってもよい。ヌクレオチド類似体の例としては、モルホリノ（WO91/09033記載の化合物）及びペプチド核酸（PNA）が挙げられる。

【００３４】

モルホリノは、下記の部分構造を有している。モルホリノは、リボースではなく、モルホリン環である点でヌクレオチドと異なっている。モルホリン環の4’位にはメチレンが結合している。5’位は、主鎖が1～3個の原子から構成される基を介して、隣接するモルホリノの窒素原子と連結される。

【化５】

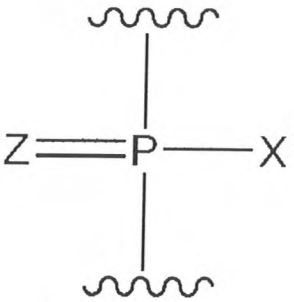


（式中、Baseは、アデニン、グアニン、ヒポキサンチン、シトシン、チミン又はウラシルのいずれかの塩基若しくは修飾塩基を示す。）

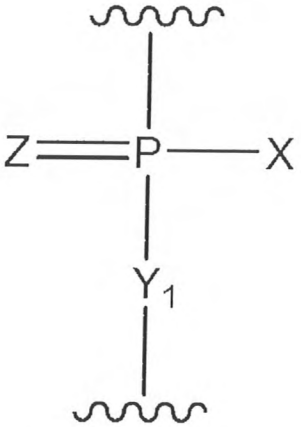
【００３５】

前記主鎖が1～3個の原子から構成される基は、以下のいずれかの式で表わされる。

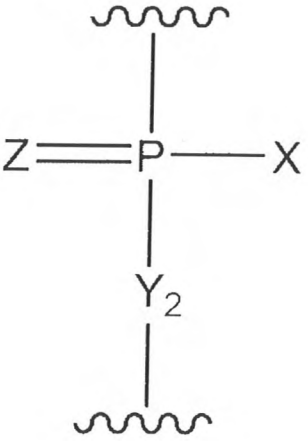
【化6】



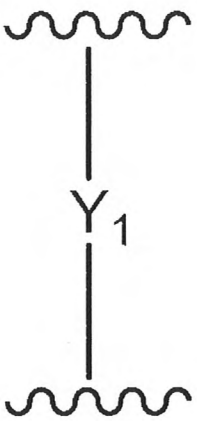
【化7】



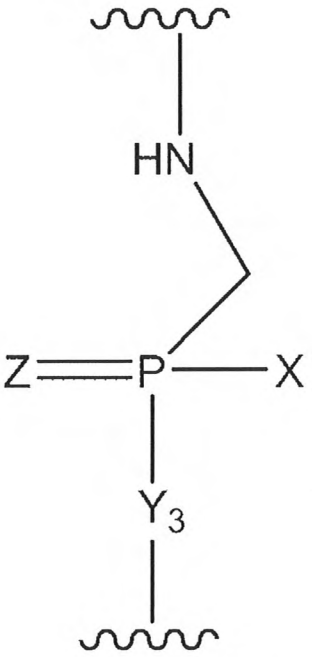
【化8】



【化９】



【化１０】



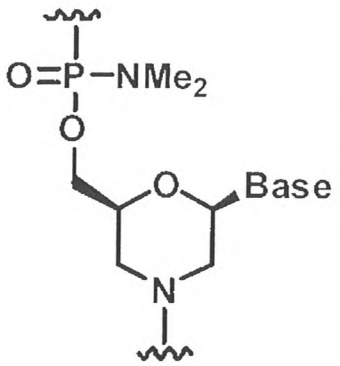
これらの結合基において、Xは、$-CH_2R^1$、$-O-CH_2R^1$、$-S-CH_2R^1$、$-NR^2R^3$又はFを表し、R^1は、H、メチル又は標的配列との結合に影響を与えない原子団であり、R^2及びR^3は、互いに異なっていてもよく、かつR^1又は環状の脂肪族基若しくは芳香族基であり、Y_1は、O、S、CH_2又はNR^1であり、Y_2は、O、S又はCH_2であり、Y_3は、O、S又はNR^1であり、Zは、O又はSである。

モルホリノの構造の詳細については、国際公開公報第1991/009033号を参照することができる。

【００３６】

ある態様において、本発明のオリゴマーのモノマーは、以下の式で表わされるホスホロジアミデートである。

【化１１】



（式中、Meは、メチルを示し、Baseは、アデニン、グアニン、ヒポキサンチン、シトシン、チミン又はウラシルのいずれかの塩基若しくは修飾塩基を示す。）

【００３７】

ホスホロジアミデートモルホリノオリゴマー（PMO）は、そのモノマーが、上記構造を有しているため、他のホスホロジアミデートモルホリノ鎖又は天然のヌクレオチド鎖とWatson-Crick塩基対を形成することが可能である（Corey, D. R. and Abrams, J. M. (2001) Genome Biol. 2, reviews 1015.1-1015.3）。PMOは、長時間にわたって細胞内でアンチセンス効果を維持することが可能であり、また、PMOは、リン酸基の-O-が、NMe_2で置換され、電気的に中性であるため、標的遺伝子以外の生体分子と非特異的結合を起こしにくいという利点がある。PMOの合成方法については、以下の文献を参照できる：国際公開公報WO2009/064471。

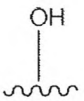
【００３８】

本発明のオリゴマーにおいて、塩基は、天然の塩基（アデニン、グアニン、ヒポキサンチン、シトシン、チミン又はウラシル）であってもよく、又は修飾塩基であってもよい。修飾塩基としては、例えば、ピリジン-4-オン、ピリジン-2-オン、フェニル、シュードウラシル、2,4,6-トリメトキシベンゼン、3-メチルウラシル。ジヒドロウリジン、ナフチル、アミノフェニル、5-アルキルシチジン（例えば、5-メチルシチジン）、5-アルキルウリジン（例えば、リボチミジン）、5-ハロウリジン（5-ブロモウリジン）、6-アザピリミジン、6-アルキルピリミジン（6-メチルウリジン）、プロピン、クエオシン（queosine）、2-チオウリジン、4-チオウリジン、ウィブトシン（wybutosine）、ウィブトキソシン（wybutoxosine）、4-アセチルチジン、5-(カルボキシヒドロキシメチル)ウリジン、5'-カルボキシメチルアミノメチル-2-チオウリジン、5-カルボキシメチルアミノメチルウリジン、β-D-ガラクトシルクエオシン（galactosylqueosine）、1-メチルアデノシン、1-メチルヒポキサンチン、2,2-ジメチルグアノシン、3-メチルシチジン、2-メチルアデノシン、2-メチルグアノシン、N6-メチルアデノシン、7-メチルグアノシン、5-メトキシアミノメチル-2-チオウリジン、5-メチルアミノメチルウリジン、5-メチルカルボニルメチルウリジン、5-メチルオキシウリジン、5-メチル-2-チオウリジン、2-メチルチオ-N6-イソペンテニルアデノシン、β-D-マンノシルクエオシン、ウリジン-5-オキシ酢酸、2-チオシチジン、トレオニン誘導体、プリン、2,6-ジアミノプリン、2-アミノプリン、イソグアニン、インドール、イミダゾール、キサンチン等が挙げられるが、これらに限定されるものではない。

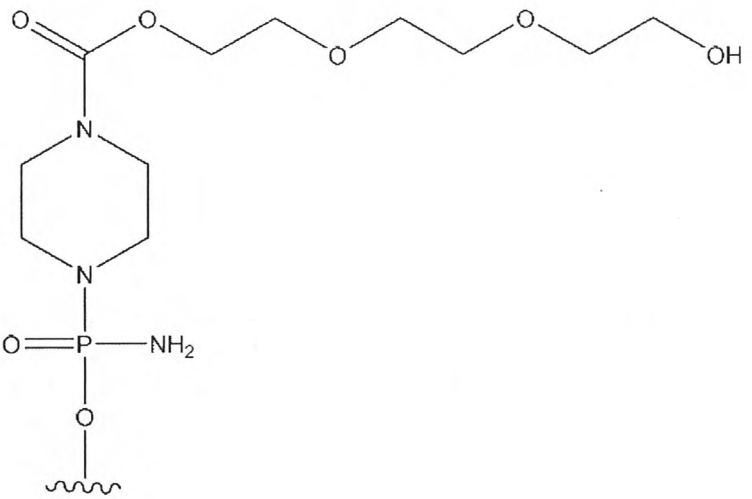
【００３９】

また、本発明のオリゴマーは、5'末端核酸残基中のリボース又はモルホリノ環に結合した5'メチレンが、下記のいずれかの基により修飾されていてもよい。

【化１２】

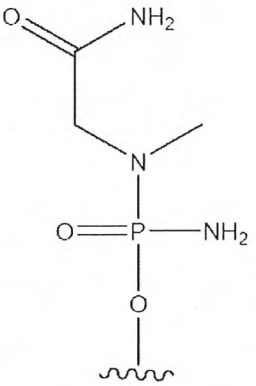


【化１３】



、又は

【化１４】



【００４０】

2．医薬組成物

本発明のオリゴマーは、従来技術に係るアンチセンスオリゴマーと比較して、高効率にエクソン53のスキッピングを可能にする。従って、本発明のオリゴマーを含む医薬組成物をDMD患者に投与することにより、高効率に筋ジストロフィーの症状を緩和することができると予測される。例えば、本発明のオリゴマーを含む医薬組成物を用いる場合、従来技術に係るオリゴマーと比べて少量の投与量でも同程度の治療効果を得られるため、副作用を軽減することができ、かつ経済的である。

そこで、別の実施態様として、本発明のオリゴマー、その医薬的に許容可能な塩又は水和物を有効成分とする、筋ジストロフィー治療用医薬組成物（以下、「本発明の組成物」という）を提供する。

【００４１】

本発明の組成物に含まれるオリゴマーの医薬的に許容可能な塩の例としては、ナトリウム塩、カリウム塩、リチウム塩のようなアルカリ金属塩、カルシウム塩、マグネシウム塩のようなアルカリ土類金属塩；アルミニウム塩、鉄塩、亜鉛塩、銅塩、ニッケル塩、コバルト塩などの金属塩;アンモニウム塩のような無機塩；t-オクチルアミン塩、ジベンジルアミン塩、モルホリン塩、グルコサミン塩、フェニルグリシンアルキルエステル塩、エチレンジアミン塩、N-メチルグルカミン塩、グアニジン塩、ジエチルアミン塩、トリエチルアミン塩、ジシクロヘキシルアミン塩、N, N' -ジベンジルエチレンジアミン塩、クロロプロカイン塩、プロカイン塩、ジエタノールアミン塩、N-ベンジル-フェネチルアミン塩、ピペラジン塩、テトラメチルアンモニウム塩、トリス(ヒドロキシメチル)アミノメタン塩のような有機塩などのアミン塩;弗化水素酸塩、塩酸塩、臭化水素酸塩、沃化水素酸塩のようなハロゲン化水素酸塩；硝酸塩、過塩素酸塩、硫酸塩、リン酸塩などの無機酸塩;メタンスルホン酸塩、トリフルオロメタンスルホン酸塩、エタンスルホン酸塩のような低級アルカンスルホン酸塩；ベンゼンスルホン酸塩、p-トルエンスルホン酸塩のようなアリールスルホン酸塩；酢酸塩、りんご酸塩、フマール酸塩、コハク酸塩、クエン酸塩、酒石酸塩、諺酸塩、マレイン酸塩などの有機酸塩;グリシン塩、リジン塩、アルギニン塩、オルニチン塩、グルタミン酸塩、アスパラギン酸塩のようなアミノ酸塩などが挙げられる。これらの塩は、公知の方法で製造することができる。あるいは、本発明の組成物に含まれるオリゴマーは、その水和物の形態にあってもよい。

【００４２】

本発明の組成物の投与形態は、医薬的に許容可能な投与形態であれば特に制限されず、治療方法に応じて選択することができるが、筋組織への送達容易性の観点から、静脈内投与、動脈内投与、筋肉内投与、皮下投与、経口投与、組織内投与、経皮投与等が好ましい。また、本発明の組成物が取り得る剤型としては、特に制限されないが、例えば、各種の注射剤、経口剤、点滴剤、吸入剤、軟膏剤、ローション剤等を挙げることができる。

【００４３】

本発明のオリゴマーを筋ジストロフィー患者に投与する場合、本発明の組成物は、該オリゴマーの筋組織への送達を促進する担体を含むことが好ましい。このような担体は、医薬的に許容可能なものであれば特に制限されず、その例として、カチオン性リポソーム、カチオン性ポリマー等のカチオン性担体、またはウイルスエンベロープを利用した担体を挙げることができる。カチオン性リポソームとしては、例えば、2-O-（2-ジエチルアミノエチル）カルバモイル-1,3-O-ジオレオイルグリセロールとリン脂質とを必須構成成分として形成されるリポソーム（以下、「リポソームA」という）、オリゴフェクトアミン（登録商標）（Invitrogen社製）、リポフェクチン（登録商標）（Invitrogen社製）、リポフェクトアミン（登録商標）（Invitrogen社製）、Lipofectamine 2000（登録商標）（Invitrogen社製）、DMRIE-C（登録商標）（Invitrogen社製）、GeneSilencer（登録商標）（Gene Therapy Systems社製）、TransMessenger（登録商標）（QIAGEN社製）、TransIT TKO（登録商標）（Mirus社製）、Nucleofector II（Lonza）を挙げることができる。それらの中で、リポソームAが好ましい。カチオン性ポリマーとしては、例えば、JetSI（登録商標）（Qbiogene社製）、Jet-PEI（登録商標）（ポリエチレンイミン、Qbiogene社製）を挙げることができる。ウイルスエンベロープを利用した担体としては、例えば、Genome One（登録商標）（HVJ-Eリポソーム、石原産業社製）を挙げることができる。あるいは、特許2924179号に記載の医薬デバイス、特許再公表公報第2006/129594号及び特許再公表公報第2008/096690号に記載のカチオン性担体を用いることもできる。

【００４４】

本発明の組成物に含まれるオリゴマーの濃度は、担体の種類等によって異なるが、0.1 nM～100 μMの範囲内が適当であり、1 nM～10 μMの範囲内が好ましく、10 nM～1 μMの範囲内がより好ましい。また、本発明の組成物に含まれるオリゴマーと担体との重量比（担体/オリゴマー）は、該オリゴマーの性質及び該担体の種類等によって異なるが、0.1～100の範囲内が適当であり、1～50の範囲内が好ましく、10～20の範囲内がより好ましい。

【００４５】

本発明の組成物には、本発明のオリゴマーと上述した担体以外に、任意に医薬的に許容可能な添加剤を配合することができる。かかる添加剤として、例えば、乳化補助剤（例えば、炭素数6～22の脂肪酸やその医薬的に許容可能な塩、アルブミン、デキストラン）、安定化剤（例えば、コレステロール、ホスファチジン酸）、等張化剤（例えば、塩化ナトリウム、グルコース、マルトース、ラクトース、スクロース、トレハロース）、pH調整剤（例えば、塩酸、硫酸、リン酸、酢酸、水酸化ナトリウム、水酸化カリウム、トリエタノールアミン）を挙げることができる。これらを一種又は二種以上使用することができる。本発明の組成物中の当該添加剤の含有量は、90重量％以下が適当であり、70重量％以下が好ましく、50重量％以下がより好ましい。

【００４６】

本発明の組成物は、担体の分散液に本発明のオリゴマーを加え、適当に攪拌することにより調製することができる。また、添加剤は、本発明のオリゴマーの添加前でも添加後でも適当な工程で添加することができる。本発明オリゴ二本鎖RNAのオリゴマーを添加させる際に用い得る水性溶媒としては、医薬的に許容可能なものであれば特に制限されず、例えば、注射用水、注射用蒸留水、生理食塩水等の電解質液、ブドウ糖液、マルトース液等の糖液を挙げることができる。また、かかる場合のpH及び温度等の条件は、当業者が適宜選択することができる。

【００４７】

本発明の組成物は、例えば、液剤やその凍結乾燥製剤とすることができる。当該凍結乾燥製剤は、常法により、液剤の形態を有している本発明の組成物を凍結乾燥処理することにより調製することができる。例えば、液剤の形態を有している本発明の組成物を適当な滅菌を行った後、所定量をバイアル瓶に分注し、約－40～－20℃の条件で予備凍結を2時間程度行い、約0～10℃で減圧下に一次乾燥を行い、次いで、約15～25℃で減圧下に二次乾燥して凍結乾燥することができる。そして、一般的にはバイアル内部を窒素ガスで置換し、打栓して本発明の組成物の凍結乾燥製剤を得ることができる。

【００４８】

本発明の組成物の凍結乾燥製剤は、一般には任意の適当な溶液（再溶解液）の添加によって再溶解し使用することができる。このような再溶解液としては、注射用水、生理食塩水、その他一般輸液を挙げることができる。この再溶解液の液量は、用途等によって異なり特に制限されないが、凍結乾燥前の液量の0.5～2倍量、又は500 mL以下が適当である。

【００４９】

本発明の組成物を投与する際の用量としては、含有される本発明のオリゴマーの種類、剤型、年齢や体重等の患者の状態、投与経路、疾患の性質と程度を考慮した上で調製することが望ましいが、成人に対して本発明のオリゴマーの量として、1日当たり0.1mg～10g/ヒトの範囲内が、好ましくは1 mg～1 gの範囲内が一般的である。この数値は標的とする疾患の種類、投与形態、標的分子によっても異なる場合がある。従って、場合によってはこれ以下でも十分であるし、また逆にこれ以上の用量を必要とするときもある。また1日1回から数回の投与又は1日から数日間の間隔で投与することができる。

【００５０】

本発明の組成物の別の態様として、本発明のオリゴヌクレオチドを発現し得るベクターと上述した担体とを含む医薬組成物を挙げることができる。かかる発現ベクターは、複数の本発明のオリゴヌクレオチドを発現し得るものであってもよい。当該組成物には、本発明のオリゴマーを含有する本発明の組成物と同様に、医薬的に許容可能な添加剤を添加することができる。当該組成物中に含まれる発現ベクターの濃度は、担体の種類等によって異なるが、0.1 nM～100 μMの範囲内が適当であり、1 nM～10 μMの範囲内が好ましく、10 nM～1 μMの範囲内がより好ましい。当該組成物中に含まれる発現ベクターと担体との重量比（担体/発現ベクター）は、発現ベクターの性質、担体の種類等によって異なるが、0.1～100の範囲内が適当であり、1～50の範囲内が好ましく、10～20の範囲内がより好ましい。また、当該組成物中に含まれる担体の含有量は、本発明のオリゴマーを含有する本発明の組成物の場合と同様であり、その調製方法等に関しても、本発明の組成物の場合

と同様である。

【００５１】

以下に、実施例及び試験例を掲げて、本発明をさらに詳しく説明するが、本発明は実施例に示される範囲に限定されるものではない。

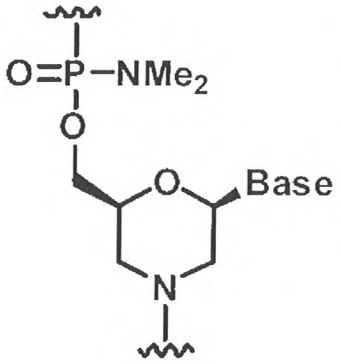
【実施例】

【００５２】

[合成例1]

国際公開公報WO2009/064471の記載に従い、AKTA oligopilot plus 10（GE Healthcare）を用いて、以下の表2に示す各種モルホリノオリゴマー（PMO）を合成した。合成したモルホリノオリゴマーを注射用水（大塚製薬工場社製）で溶解した。モルホリノオリゴマーのモノマーは、次の通りである。

【化１５】



（Meは、メチルである。）

【表２】

PMO No.	エクソン53中の標的配列	備考	PMOの配列
1	第31～55番目	5’末端修飾基：-OH	配列番号4
2	第32～53番目	5’末端修飾基：-OH	配列番号8
3	第32～56番目	5’末端修飾基：-OH	配列番号11
4	第33～54番目	5’末端修飾基：-OH	配列番号15
5	第34～58番目	5’末端修飾基：-OH	配列番号25
6	第36～55番目	5’末端修飾基：-OH	配列番号34
7	第36～53番目	5’末端修飾基：-OH	配列番号32
8	第36～55番目	5’末端修飾基：-OH	配列番号34
9	第36～56番目	5’末端修飾基：-OH	配列番号35
10	第36～57番目	5’末端修飾基：-OH	配列番号36
11	第33～57番目	5’末端修飾基：-OH	配列番号18
12	第39～69番目	非特許文献３のH53A(+39+69)（Table 1参照）に相当する配列	配列番号38
13	第30～59番目	非特許文献５のh53A30/1（Table 1参照）に相当する配列	配列番号39

【００５３】

[試験例1]

In vitroアッセイ

RD細胞（ヒト横紋筋肉腫細胞株）4×10^{5}個に対して、No. 1～9及び12のアンチセンス10μMをAmaxa Cell Line Nucleofector Kit Lを用いてNucleofector II（Lonza）により導入した。プログラムはT-030を用いた。

【００５４】

導入後、細胞を、10％ウシ胎児仔血清（FCS）（インビトロジェン社製）を含むEagle's minimal essential medium(EMEM)培地（シグマ社製、以下同じ。） 2mL中、37℃、5%CO$_2$条件下で一晩培養した。細胞をPBS（ニッスイ社製、以下同じ。）で2回洗浄した後、ISOGEN （ニッポンジーン社製）500 μl を細胞に添加し、数分間室温に放置して細胞を溶解させ、該溶解物をEppendorfチューブに回収した。ISOGENに添付のプロトコールに従ってtotal RNAを抽出した。抽出したtotal RNAの濃度はNanoDrop ND-1000（エル・エム・エス社製）を用いて測定した。

【００５５】

抽出したtotal RNA 400 ngに対し、Titan One Tube RT-PCR Kit（ロシュ社製）を用いてOne-Step RT-PCRを行った。キットに添付のプロトコールに従って、反応液を調製した。サーマルサイクラーはPTC-100（MJ Research社製）を用いた。用いたRT-PCRのプログラムは、以下の通りである。

50℃、30分間：逆転写反応

94℃、2分間：熱変性
[94℃、10秒間；58℃、30秒間；68 ℃、45秒間] x 30サイクル：PCR増幅
68℃、7分間：ポリメラーゼの熱失活

【００５６】
RT-PCRに使用したフォワードプライマーとリバースプライマーの塩基配列は以下の通りである。

フォワードプライマー：5’-AGGATTTGGAACAGAGGCGTC-3’　（配列番号40）
リバースプライマー：5’-GTCTGCCACTGGCGGAGGTC-3’　（配列番号41）

【００５７】
次に、上記RT-PCRの増幅産物に対し、Taq DNA Polymerase（ロシュ社製）を用いてnested PCRを行った。用いたPCRプログラムは、以下の通りである。

94℃、2分間：熱変性
[94℃、15秒間；58℃、30秒間；68 ℃、45秒間] x 30サイクル：PCR増幅
68℃、7分間：ポリメラーゼの熱失活

【００５８】
上記nested PCRに使用したフォワードプライマーとリバースプライマーの塩基配列は以下の通りである。

フォワードプライマー：5’-CATCAAGCAGAAGGCAACAA-3’　（配列番号42）
リバースプライマー：5’-GAAGTTTCAGGGCCAAGTCA-3’　（配列番号43）

【００５９】
上記nested PCRの反応産物1 μlをBioanalyzer（アジレント社製）を用いて解析した。
エクソン53 がスキップしたバンドのポリヌクレオチド量「A」と、エクソン53がスキップしなかったバンドのポリヌクレオチド量「B」を測定した。これら「A」及び「B」の測定値に基づき、以下の式に従って、スキッピング効率を求めた。

スキッピング効率（%）= A / (A + B) x 100

【００６０】
実験結果
結果を図1に示す。本実験により、No. 1～9のアンチセンスオリゴマーは、いずれもNo. 12のアンチセンスオリゴマーと比べて、著しく高い効率でエクソン53をスキッピングさせることが判明した。特に、No. 3及び9のアンチセンスオリゴマーは、No. 12のアンチセンスオリゴマーと比べて、4倍以上高いエクソンスキッピング効率を示す。

【００６１】
[試験例2]
ヒト線維芽細胞を用いたIn vitroアッセイ
ZsGreen1共発現レトロウイルスベクターによりTIG-119細胞（ヒト正常組織由来線維芽

細胞、医薬基盤研究所）又は5017細胞（ヒトDMD患者由来線維芽細胞、Coriell Institute for Medical Research）にヒトmyoD遺伝子（配列番号44）を導入した。

【００６２】
4から5日間インキュベートした後に、FACSによりZsGreen陽性のMyoD転換線維芽細胞を回収し、5×10^{4}個/cm^{2}になるように12穴プレートに播種した。増殖培地は10％FCS及び1％Penicillin/Streptomycin（P/S）（シグマ　アルドリッチ社）を含むDulbecco's Modified Eagle Medium:Nutrient Mixture F-12（DMEM・F-12）（インビトロジェン社）を1 mL使用した。

【００６３】
24時間後に分化培地（2％ウマ血清（インビトロジェン社）、1％P/S及びITS Liquid Media Supplement（シグマ社）含有DMEM/F-12）に交換した。2、3日ごとに培地交換を行い12から14日間インキュベートし、筋管細胞に分化させた。

【００６４】
その後、分化培地を6 μMのEndo-Porter（ジーンツール社）含有分化培地に交換し、終濃度10μMになるようにモルホリノオリゴマーを添加した。48時間インキュベート後に、TRIzol（インビトロジェン社製）により、細胞からtotal RNAを抽出した。抽出したtotal RNA 50 ngに対し、QIAGEN OneStep RT-PCR Kitを用いてRT-PCRを行った。添付のプロトコールに従って、反応液を調製した。サーマルサイクラーはiCycler（Bio-Rad社製）を用いた。用いたRT-PCRのプログラムは、以下の通りである。

50℃、30分間：逆転写反応
95℃、15分間：熱変性
[94℃、1分間；60℃、1分間；72 ℃、1分間] x 35サイクル：PCR増幅
72℃、7分間：ポリメラーゼの熱失活

【００６５】
プライマーはhEX51F及びhEX55Rを使用した。

hEX51F：5’-CGGGCTTGGACAGAACTTAC-3’　（配列番号45）
hEx55R：5’-TCCTTACGGGTAGCATCCTG-3’　（配列番号46）

【００６６】
上記RT-PCR反応の反応産物を2％アガロースゲル電気泳動によって分離し、GeneFlash（Syngene社）によりゲル写真を撮影した。Image J（アメリカ国立衛生研究所製）により、エクソン53 がスキップしたバンドのポリヌクレオチド量「A」と、エクソン53がスキップしなかったバンドのポリヌクレオチド量「B」を測定した。これら「A」及び「B」の測定値に基づき、以下の式に従って、スキッピング効率を求めた。

スキッピング効率（％）= A /（A ＋ B）x 100

【００６７】
実験結果
結果を図2及び図3に示す。本実験により、No. 3、9及び10のアンチセンスオリゴマー（図2）は、TIG-119細胞において、いずれもNo. 13のアンチセンスオリゴマーと比べて、高

い効率でエクソン53をスキッピングさせることが判明した（図2）。特に、No. 3及び9のアンチセンスオリゴマーは、No. 13のアンチセンスオリゴマーと比べて、2倍以上高いエクソンスキッピング効率を示す（図2）。

また、本実験により、No. 3及び9～11のアンチセンスオリゴマー（図3）は、5017細胞において、いずれもNo. 13のアンチセンスオリゴマーと比べて、高い効率でエクソン53をスキッピングさせることが判明した（図3）。特に、No. 3及び9のアンチセンスオリゴマーは、No. 13のアンチセンスオリゴマーと比べて、7倍以上高いエクソンスキッピング効率を示す（図3）。

【産業上の利用可能性】

【００６８】

実施例に示す実験結果から、本発明のオリゴマー（No. 1～11）は、従来技術に係るオリゴマー（No. 12及び13）と比べ、いずれの細胞環境においても、著しく高い効率でエクソン53をスキッピングさせることが示された。また、実施例2で用いた5017細胞は、デュシェンヌ型筋ジストロフィー患者から採取した細胞であるため、本発明のオリゴマーは、実際にデュシェンヌ型筋ジストロフィー患者に投与した場合にも、高効率にエクソン53をスキッピングさせると考えられる。

従って、本発明のオリゴマーは、デュシェンヌ型筋ジストロフィーの治療において、非常に有用である。

【配列表フリーテキスト】

【００６９】

配列番号２：合成核酸
配列番号３：合成核酸
配列番号４：合成核酸
配列番号５：合成核酸
配列番号６：合成核酸
配列番号７：合成核酸
配列番号８：合成核酸
配列番号９：合成核酸
配列番号１０：合成核酸
配列番号１１：合成核酸
配列番号１２：合成核酸
配列番号１３：合成核酸
配列番号１４：合成核酸
配列番号１５：合成核酸
配列番号１６：合成核酸
配列番号１７：合成核酸
配列番号１８：合成核酸
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配列番号２１：合成核酸
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配列番号２３：合成核酸
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配列番号２５：合成核酸
配列番号２６：合成核酸
配列番号２７：合成核酸
配列番号２８：合成核酸
配列番号２９：合成核酸
配列番号３０：合成核酸
配列番号３１：合成核酸

配列番号３２：合成核酸
配列番号３３：合成核酸
配列番号３４：合成核酸
配列番号３５：合成核酸
配列番号３６：合成核酸
配列番号３７：合成核酸
配列番号３８：合成核酸
配列番号３９：合成核酸
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配列番号４１：合成核酸
配列番号４２：合成核酸
配列番号４３：合成核酸
配列番号４５：合成核酸
配列番号４６：合成核酸

【配列表】

SEQUENCE LISTING

<110> NIPPON SHINYAKU CO., LTD.
NATIONAL CENTER OF NEUROLOGY AND PSYCHIATRY

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tgcaagcgca agaccaccaa cgccgaccgc cgcaaggccg ccaccatgcg cgagcggcgc 360

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aaccagcggt tgcccaaggt ggagatcctg cgcaacgcca tccgctatat cgagggcctg 480

caggctctgc tgcgcgacca ggacgccgcg ccccctggcg ccgcagccgc cttctatgcg 540

ccgggccccgc tgccccccggg ccgcggcggc gagcactaca gcggcgactc cgacgcgtcc 600

agcccgcgct ccaactgctc cgacggcatg atggactaca gcggcccccc gagcggcgcc 660

cggcggcgga actgctacga aggcgcctac tacaacgagg cgcccagcga acccaggccc 720

gggaagagtg cggcggtgtc gagcctagac tgcctgtcca gcatcgtgga gcgcatctcc 780

accgagagcc ctgcggcgcc cgccctcctg ctggcggacg tgccttctga gtcgcctccg 840

cgcaggcaag aggctgccgc ccccagcgag ggagagagca gcggcgaccc cacccagtca 900

ccggacgccg ccccgcagtg ccctgcgggt gcgaacccca acccgatata ccaggtgctc 960

tga 963

<210> 45
<211> 20
<212> DNA
<213> Artificial

<220>
<223> Synthetic Nucleic Acid

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cgggcttgga cagaacttac 20

<210> 46
<211> 20
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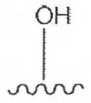
<220>
<223> Synthetic Nucleic Acid

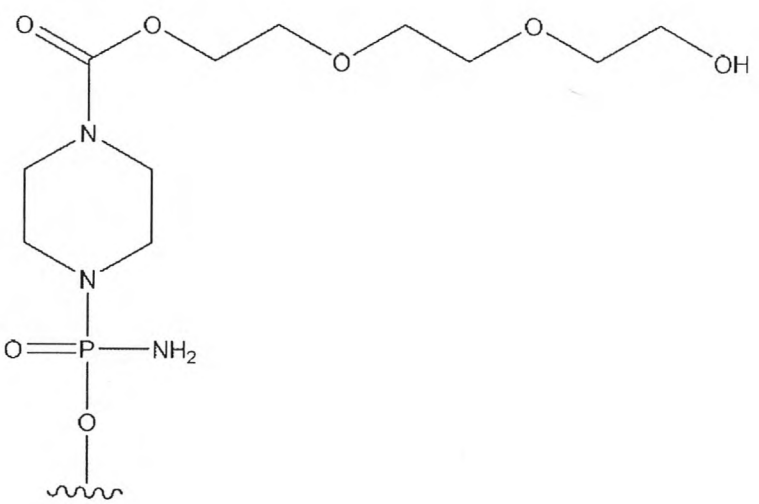
<400> 46
tccttacggg tagcatcctg 20

【書類名】特許請求の範囲
【請求項１】
ヒトジストロフィン遺伝子の第53番目のエクソンのスキッピングを可能にするアンチセンスオリゴマーであって、ヒトジストロフィン遺伝子の第53番エクソンの5’末端から第31～53番目、第31～54番目、第31～55番目、第31～56番目、第31～57番目、第31～58番目、第32～53番目、第32～54番目、第32～55番目、第32～56番目、第32～57番目、第32～58番目、第33～53番目、第33～54番目、第33～55番目、第33～56番目、第33～57番目、第33～58番目、第34～53番目、第34～54番目、第34～55番目、第34～56番目、第34～57番目、第34～58番目、第35～53番目、第35～54番目、第35～55番目、第35～56番目、第35～57番目、第35～58番目、第36～53番目、第36～54番目、第36～55番目、第36～56番目、第36～57番目又は第36～58番目のヌクレオチドからなる配列のいずれか1つに相補的な塩基配列からなる、アンチセンスオリゴマー。
【請求項２】
オリゴヌクレオチドである、請求項１に記載のアンチセンスオリゴマー。
【請求項３】
前記オリゴヌクレオチドを構成する糖及び/又はリン酸の少なくとも１つが修飾されている、請求項2に記載のアンチセンスオリゴマー。
【請求項４】
前記オリゴヌクレオチドを構成する糖の2’位の水酸基が、OR、R、R’OR、SH、SR、NH_2、NHR、NR_2、N_3、CN、F、Cl、Br及びIからなる群より選択されるいずれかの基で置換された、請求項3に記載のアンチセンスオリゴマー。
（上記Rは、炭素数1～6のアルキル又は炭素数1～6のアリールを示し、上記R’は、炭素数1～6のアルキレンを示す。）
【請求項５】
前記オリゴヌクレオチドを構成するリン酸が、ホスホロチオエート、ホスホロジチオエート、アルキルホスホネート及びホスホロアミデートからなる群より選択されるいずれか1つのものである、請求項3又は4に記載のアンチセンスオリゴマー。
【請求項６】
モルホリノオリゴマーである、請求項1に記載のアンチセンスオリゴマー。
【請求項７】
ホスホロジアミデートモルホリノオリゴマーである、請求項６に記載のアンチセンスオリゴマー。
【請求項８】
5’末端核酸残基のリボース又はモルホリノ環に結合した5’メチレンが、下記のいずれかの基により修飾されている、請求項1～7のいずれか1項に記載のアンチセンスオリゴマー。

【化１６】

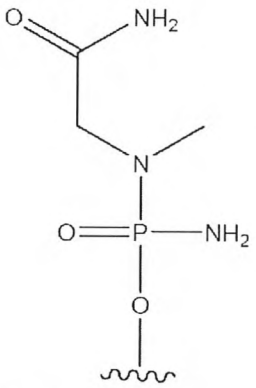


【化１７】



、又は

【化１８】



【請求項９】
ヒトジストロフィン遺伝子の第53番エクソンの5’末端から第32～56番目又は第36～56番目のヌクレオチドからなる配列に相補的な塩基配列からなる、請求項1～8のいずれか1項に記載のアンチセンスオリゴマー。
【請求項１０】
配列番号2～37からなる群より選択されるいずれか1つに示す塩基配列からなる、請求項1～8のいずれか1項に記載のアンチセンスオリゴマー。
【請求項１１】
配列番号11、17、23、29及び35からなる群より選択されるいずれか1つに示す塩基配列からなる、請求項1～8のいずれか1項に記載のアンチセンスオリゴマー。
【請求項１２】
配列番号11又は35に示す塩基配列からなる、請求項1～8のいずれか1項に記載のアンチセンスオリゴマー。
【請求項１３】
請求項1～12のいずれか1項に記載のアンチセンスオリゴマー、その医薬的に許容可能な塩又は水和物を有効成分とする、筋ジストロフィー治療用医薬組成物。

【書類名】要約書
【要約】
【課題】　ヒトジストロフィン遺伝子の第53番目のエクソンを、高効率にスキッピングさせる薬剤の提供。
【解決手段】
本発明は、ヒトジストロフィン遺伝子の第53番目のエクソンのスキッピングを可能にするオリゴマーを提供する。
【選択図】なし

【書類名】図面
【図１】

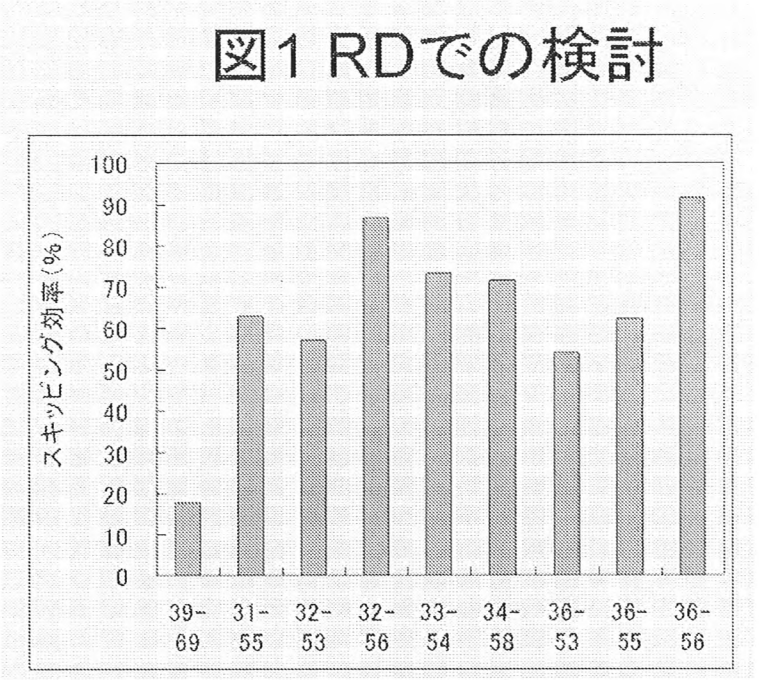


図1 RDでの検討
スキッピング効率（%）
100
90
80
70
60
50
40
30
20
10
0
39-69
31-55
32-53
32-56
33-54
34-58
36-53
36-55
36-56

【図２】

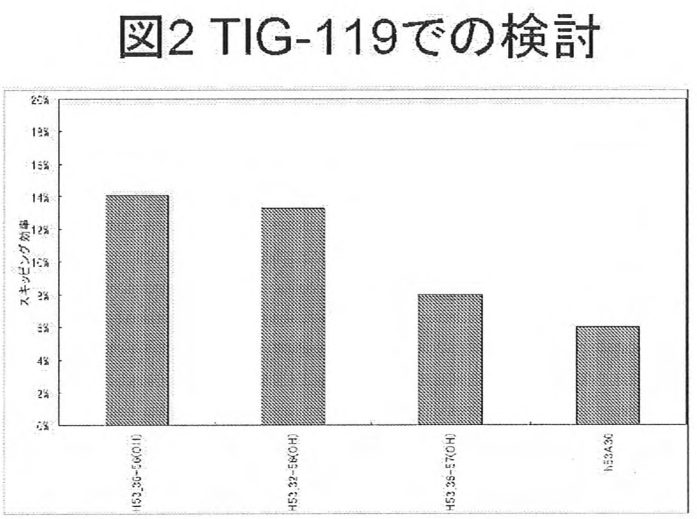


図2 TIG-119での検討

【図３】

図3 5017での検討

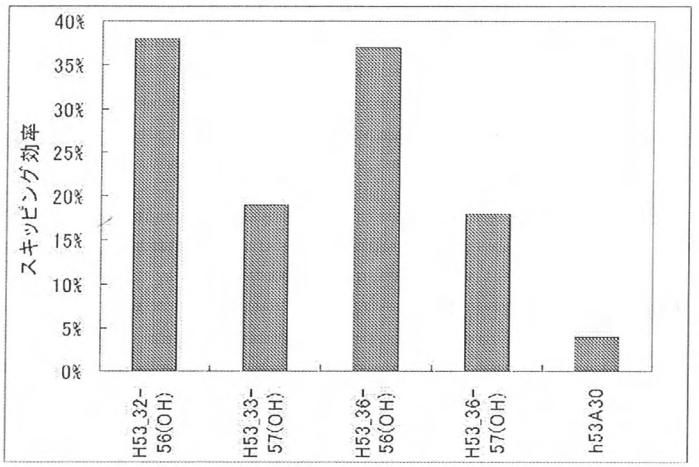


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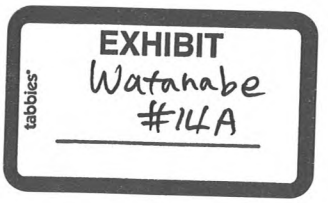
EXHIBIT AH

JAPAN PATENT OFFICE

This is to certify that the annexed is a true copy of the following application as filed with this Office.

Date of Application:	1 September 2010
Application Number:	JP 2010-196032
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is	JP 2010-196032
Applicant(s):	NIPPON SHINYAKU CO., LTD. NATIONAL CENTER OF NEUROLOGY AND PSYCHIATRY

20 September 2011
Iwai Yoshiyuki
Commissioner, Japan Patent Office



[Official Seal]

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[Date Submitted]	1 September 2010
[To]	Commissioner, Patent Office
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[Prepaid Docket No.] 157061
[Value] 15,000 yen
[Items Submitted]
[Item] Claims 1
[Item] Specification 1
[Item] Abstract 1
[Item] Drawings 1

[Document Name] Specification

[Title of the invention] Antisense nucleic acid

[Technical Field]

[0001]

The present invention relates to antisense oligomers which enable skipping of exon 53 in the human dystrophin gene, and to pharmaceutical compositions which include said oligomers.

[Background Art]

[0002]

Duchenne muscular dystrophy (DMD) is the most frequent form of muscle atrophy, affecting one in ca 3500 newborn males. Although motor functions are subtantially unchanged from those of healthy humans in infancy, muscle weakness is observed from around 4-5 years old. Muscle weakness subsequently progresses, with inability to walk by about 12 years old, and death due to cardiac or respiratory insufficiency in the twenties; it is a serious disorder. There is currently no effective therapy for DMD, and the development of a new therapy is strongly desired.

[0003]

DMD is known to be caused by a mutation of the dystrophin gene. The dystrophin gene is located on the X chromosome and is a gigantic gene consisting of DNA of 2.2 million nucleotide pairs. mRNA with 79 linked exons is formed by transcription from DNA to an mRNA precursor, and then removal of introns by splicing comprises 11,058 nucleotides. This mRNA is translated into 3685 amino acids, to produce the dystrophin protein. The dystrophin protein contributes to the maintenance of membrane stability in muscle cells and is necessary to make muscle cells less fragile. Because the dystrophin gene from patients with DMD contains a mutation, there is hardly any expression of functional dystrophin protein in muscle cells. Therefore, in the bodies of DMD patients the structure of muscle cells cannot be maintained, and a large quantity of calcium ions flows into the muscle cells. As a result, an inflammation-like response occurs, promoting fibrosis so that the muscle cells cannot readily be regenerated.

[0004]

Becker muscular dystrophy (BMD) is also caused by a mutation of the dystrophin gene, but although the condition presents muscle weakness due to muscle atrophy, it is generally milder than DMD and muscle weakness also progresses more slowly; and in many cases, onset is in adulthood. It is thought that the differences in clinical symptoms between DMD and BMD depend on whether the reading frame for amino acids when dystrophin mRNA is translated into protein is disrupted due to the mutation, or is maintained (Non-Patent Document 1). Thus, in DMD there is hardly any expression of functional dystrophin protein because there is a mutation which shifts the amino acid reading frame; but in BMD, although some exons are lost due to the mutation, incomplete but functional dystrophin protein is produced, because the amino acid reading frame is maintained.

[0005]

Exon skipping offers expectations as a method for treating DMD. This method restores the amino acid reading frame of dystrophin mRNA by modifying splicing, and induces expression of protein with partially restored function (Non-Patent Document 2). The mutation and the partial amino acid sequence which is a target for exon skipping are lost. Consequently, dystrophin protein expressed by this treatment is shorter than the normal protein, but because the amino acid reading frame is maintained, it partially retains the function of stabilizing muscle cells. Therefore, it is expected that exon skipping will give DMD which presents similar symptoms to that of the milder BMD. The exon skipping approach has passed through animal experiments using mice and dogs, and clinical trials on human DMD patients are in progress.

[0006]

Exon skipping can be induced by binding of antisense nucleic acid targeting either the 5′ or 3′ splice site or both, or an exon-internal sequence. An exon will only be included in the mRNA when both splice sites are recognized by the spliceosome complex. Therefore, exon skipping can be induced by targeting a splice site with antisense nucleic acid. Binding of an SR protein to an exon splicing enhancer (ESE) is also thought to be necessary for recognition of an

exon in the splicing mechanism, and exon skipping can also be induced by targeting the ESE.

[0007]

Because mutations of the dystrophin gene differ among DMD patients, antisense nucleic acid suited to the site or type of gene mutation is needed. So far, antisense nucleic acids that induce exon skipping have been produced by Steve Wilton *et al.* of the University of Western Australia for all 79 exons (Non-Patent Document 3); and antisense nucleic acids which induce exon skipping have been produced by Annemieke Aartsma-Rus *et al.* in the Netherlands for 39 exons (Non-Patent Document 4).

[0008]

It is thought that about 8% of all DMD patients could be treated by skipping the 53rd exon (hereinafter referred to as "exon 53"). In recent years, several research organizations have reported studies on exon 53 of the dystrophin gene as a target for exon skipping (Patent Documents 1-3; Non-Patent Document 5). However, a technique for highly efficient skipping of exon 53 has yet to be established.

[Prior Art Documents]

[Patent Documents]

[0009]

Patent Document 1: WO 2006/000057 A1

Patent Document 2: WO 2004/048570 A1

Patent Document 3: US 2010/0168212 A1

[Non-Patent Documents]

[0010]

Non-Patent Document 1: Monaco A. P. *et al.*, Genomics 1988; 2: p. 90-95

Non-Patent Document 2: Matsuo M., Brain Dev 1996; 18: p. 167-172

Non-Patent Document 3: Wilton S. D., *et al.*, Molecular Therapy 2007: 15: p. 1288-96

Non-Patent Document 4: Annemieke Aartsma-Rus *et al.*, (2002) Neuromuscular Disorders 12: S71-S77

Non-Patent Document 5: Linda J. Popplewell *et al.*, (2010) Neuromuscular Disorders , vol. 20, no. 2, p. 102-10

[Synopsis of the Invention]

[Problem which the invention is intended to solve]

[0011]

Given the situation described above, an antisense oligomer that strongly induces skipping of exon 53 of the dystrophin gene and treatments for muscular dystrophy which include such an oligomer are desired.

[Means for solving the problem]

[0012]

As a result of detailed studies of the structure of dystrophin mutant genes having a mutation in exon 53, the present inventors have found that skipping of exon 53 can be induced with high efficiency by targeting the sequence comprising peripheral nucleotides 32-56 from the 5' end of exon 53 in the mRNA precursor (hereinafter referred to as "pre-mRNA") of the dystrophin gene, by using an antisense oligomer. The present inventors have perfected the present invention based on this insight.

[0013]

Thus, the present invention is as follows.

[1] An antisense oligomer, which is an antisense oligomer which enables skipping of exon 53 of the human dystrophin gene, comprising a nucleotide sequence complementary to any one of the sequences comprising nucleotides 31-53, 31-54, 31-55, 31-56, 31-57, 31-58, 32-53, 32-54, 32-55, 32-56, 32-57, 32-58, 33-53, 33-54, 33-55, 33-56, 33-57, 33-58, 34-53, 34-54, 34-55, 34-56, 34-57, 34-58, 35-53, 35-54, 35-55, 35-56, 35-57, 35-58, 36-53, 36-54, 36-55, 36-56, 36-57 or 36-58 from the 5' end of exon 53 in the human dystrophin gene.

[2] An antisense oligomer according to [1] above, which is an oligonucleotide.

[3] An antisense oligomer according to [2] above, wherein the sugar moiety and/or the phosphate-binding moiety of at least one nucleotide constituting the oligonucleotide is modified.

[4] An antisense oligomer according to [3] above, wherein the sugar moiety of at least one nucleotide constituting the oligonucleotide is ribose in which the 2'-OH group is replaced by any group selected from a set comprising OR, R, R' OR, SH, SR, NH_2, NHR, NR_2, N_3, CN, F, Cl, Br and I. (Where R

indicates a C1-6 alkyl or C1-6 aryl and R' indicates a C1-6 alkylene.)

[5] An antisense oligomer according to [3] or [4] above, wherein the phosphate constituting the oligonucleotide is any one selected from a set comprising a phosphorothioate bond, a phosphorodithioate bond, an alkylphosphonate bond and a phosphoroamidate bond.

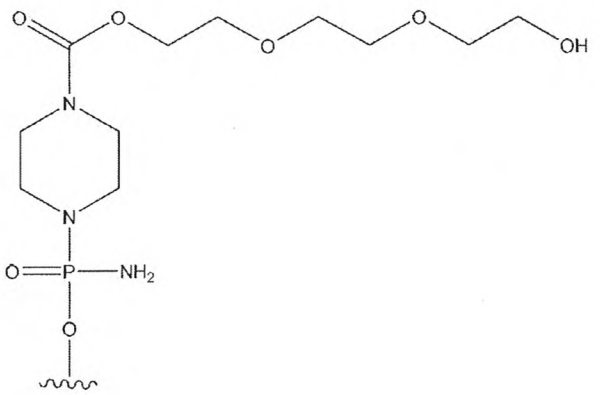
[6] An antisense oligomer according to [1] above, which is a morpholino oligomer.

[7] An antisense oligomer according to [6] above which is a phosphorodiamidate morpholino oligomer.

[8] An antisense oligomer according to any one of [1]-[7] above, wherein the 5'-methylene bound to the ribose or morpholino ring of the 5' terminal nucleic acid residue is modified with any one of the groups below.

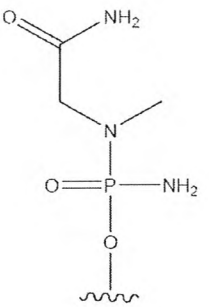
[Formula 1]

[Formula 2]



or

[Formula 3]



[9] An antisense oligomer according to any one of [1]-[8] above, comprising a nucleotide sequence complementary to a sequence comprising nucleotides 32-56 or 36-56 from the 5' end of exon 53 of the human dystrophin gene.

[10] An antisense oligomer according to any one of [1]-[8] above, comprising any one nucleotide sequence selected from a set comprising SEQ ID NO: 2-37.

[11] An antisense oligomer according to any one of [1]-[8] above, comprising any one nucleotide sequence selected from a set comprising SEQ ID NO: 11, 17, 23, 29 and 35.

[12] An antisense oligomer according to any one of [1]-[8] above, comprising the nucleotide sequence of either SEQ ID NO: 11 or 35.

[13] A pharmaceutical composition for treating muscular dystrophy, in which an active ingredient is an antisense oligomer according to any one of [1] to [12] above, or a pharmaceutically acceptable salt or hydrate thereof.

[Effects of the invention]

[0014]

An antisense oligomer of the present invention can induce skipping of exon 53 of the dystrophin gene with high efficiency. In addition, the symptoms of Duchenne muscular dystrophy can be effectively alleviated by administering a pharmaceutical composition of the present invention.

[Brief Description of the Drawings]

[0015]

[Figure 1] is a graph showing the efficiency of skipping of exon 53 of the dystrophin gene in human rhabdomyosarcoma cell line (RD cells).

[Figure 2] is a graph showing the efficiency of skipping of exon 53 of the dystrophin gene in fibroblasts from normal human tissue (TIG-119 cells), induced to differentiate into muscle cells by introducing the human myoD gene.

[Figure 3] is a graph showing the efficiency of skipping of exon 53 of the dystrophin gene in fibroblasts from a human DMD patient (5017 cells), induced to differentiate into muscle cells by introducing the human myoD gene.

[Mode for Carrying Out the Invention]

[0016]

1. Antisense oligomers

The present invention offers antisense oligomers (hereinafter referred to as "oligomers of the present invention") which enable skipping of exon 53 in the human dystrophin gene, comprising a nucleotide sequence complementary to any one of the sequences (hereinafter also referred to as "target sequences") consisting of nucleotides 31-53, 31-54, 31-55, 31-56, 31-57, 31-58, 32-53, 32-54, 32-55, 32-56, 32-57, 32-58, 33-53, 33-54, 33-55, 33-56, 33-57, 33-58, 34-53, 34-54, 34-55, 34-56, 34-57, 34-58, 35-53, 35-54, 35-55, 35-56, 35-57, 35-58, 36-53, 36-54, 36-55, 36-56, 36-57, or 36-58 from the 5' end of exon 53 of the human dystrophin gene.

[0017]

[Exon 53 of the human dystrophin gene]

In the present invention, the term "gene", in addition to genomic genes, also includes cDNA, mRNA precursors and mRNA. Preferably, the gene is an mRNA precursor, i.e., pre-mRNA.

In the human genome, the human dystrophin gene is located at locus Xp21.2. The human dystrophin gene has a size of 3.0 Mbp and is the largest of the known human genes. However, the coding region of the human dystrophin gene is a mere 14 kb, and said coding region is dispersed within the dystrophin gene as 79 exons (Roberts, RG., *et al.*, Genomics, 16: 536-538 (1993)). Pre-mRNA, which is the transcript of the human dystrophin gene, undergoes splicing to produce mature mRNA of 14 kb. The nucleotide sequence of the human wild-type dystrophin gene is known (GenBank Accession No. NM_004006).

The nucleotide sequence of exon 53 in the human wild-type dystrophin gene is shown in SEQ ID NO: 1.

[0018]

The oligomers of the present invention are created in order to modify the protein encoded by a DMD dystrophin gene into a BMD dystrophin by skipping of exon 53. Therefore, exon 53 of the dystrophin gene, which is the target of exon skipping by an oligomer of the present invention, includes mutant forms as well as the wild type.

Specifically, mutant exon 53 of human dystrophin genes is a polynucleotide described in (a) or (b) below.

(a) A polynucleotide that hybridizes under stringent conditions with a polynucleotide comprising a nucleotide sequence complementary to the nucleotide sequence of SEQ ID NO: 1; and

(b) a polynucleotide comprising a nucleotide sequence having at least 90% homology with the nucleotide sequence of SEQ ID NO: 1.

[0019]

In the present description, "polynucleotide" means DNA or RNA, but is preferably RNA.

In the present description, the term "polynucleotide that hybridizes under stringent conditions" means, for example, a polynucleotide obtained by colony hybridization, plaque hybridization or Southern hybridization, etc., using as a probe all or part of a polynucleotide consisting of a nucleotide sequence complementary to the nucleotide sequence of SEQ ID NO: 1, for example. As the hybridization method, a method described, for example, in "Sambrook & Russell, Molecular Cloning: A Laboratory Manual Vol. 3, Cold Spring Harbor, Laboratory Press 2001" or "Ausubel, Current Protocols in Molecular Biology, John Wiley & Sons 1987-1997", etc., can be employed.

[0020]

In this description, the term "stringent conditions" may be any of conditions of low stringency, moderately stringent conditions or highly stringent conditions. "Conditions of low stringency" are, for example, conditions of 5×SSC, 5×Denhardts solution, 0.5% SDS and 50% formamide, at 32°C. "Moderately stringent conditions" are, for example, conditions of 5×SSC, 5×Denhardts solution, 0.5% SDS and 50% formamide, at 42°C, or 5×SSC, 1% SDS, 50 mM Tris-HCl (pH 7.5) and 50% formamide, at 42°C. "Highly stringent conditions" are, for example, 5×SSC, 5×Denhardts solution, 0.5% SDS and 50% formamide, at 50°C, or 0.2×SSC and 0.1% SDS, at 65°C. Under these conditions, polynucleotides with high homology can be expected to be obtained more efficiently at higher temperatures. However, multiple factors, such as temperature, probe concentration, probe length, ionic strength, time and salt concentration, can be expected to affect hybridization stringency, and those

skilled in the art can achieve similar stringency by appropriate selection of these factors.

[0021]

It should be noted that when using a commercially available kit for hybridization, the Alkphos Direct Labeling and Detection System (GE Healthcare), for example, can be used. In this case, hybridized polynucleotides can be detected after incubation overnight with the labeled probe, and then washing the membrane with a primary wash buffer containing 0.1% (w/v) SDS at 55°C, in accordance with the protocol included in the kit. Alternatively, when creating a probe based on an entire or partial nucleotide sequence complementary to the nucleotide sequence of SEQ ID NO: 1, if the probe is labeled with digoxigenin (DIG) using a commercially available reagent (for example, PCR Labeling Mix (Roche Diagnostics), etc.), hybridization can be detected by using a DIG Nucleic Acid Detection Kit (Roche Diagnostics).

[0022]

Polynucleotides other than the hybridizable polynucleotides described above include polynucleotides having ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99%, ≥99.1%, ≥99.2%, ≥99.3%, ≥99.4%, ≥99.5%, ≥99.6%, ≥99.7%, ≥99.8% or ≥99.9% homology with the polynucleotide of SEQ ID NO: 1, as calculated by the homology search software BLAST, using the default parameters.

[0023]

Homology between nucleotide sequences can be determined using the algorithm BLAST (Basic Local Alignment Search Tool) by Karlin and Altschul (Proc. Natl. Acad. Sci. USA 872264-2268, 1990; Proc. Natl. Acad. Sci. USA 90: 5873, 1993). BLASTN and BLASTX programs have been developed based on the BLAST algorithm (Altschul SF, et al: J. Mol. Biol. 215: 403, 1990). When a nucleotide sequence is analyzed by using BLASTN, the parameters should be, for example, score = 100 and wordlength = 12. When BLAST and Gapped BLAST programs are used, the default parameters for each program are used.

[0024]

Examples of nucleotide sequences complementary to sequences comprising nucleotides 31-53, 31-54, 31-55, 31-56,

31-57, 31-58, 32-53, 32-54, 32-55, 32-56, 32-57, 32-58, 33-53, 33-54, 33-55, 33-56, 33-57, 33-58, 34-53, 34-54, 34-55, 34-56, 34-57, 34-58, 35-53, 35-54, 35-55, 35-56, 35-57, 35-58, 36-53, 36-54, 36-55, 36-56, 36-57 and 36-58 from 5' of exon 53, are shown in the table below.

[Table 1]

Nucleotides of exon 53	Complementary nucleotide sequence	SEQ ID NO:
31-53	5'-CCGGTTCTGAAGGTGTTCTTGTA-3'	SEQ ID NO: 2
31-54	5'-TCCGGTTCTGAAGGTGTTCTTGTA-3'	SEQ ID NO: 3
31-55	5'-CTCCGGTTCTGAAGGTGTTCTTGTA-3'	SEQ ID NO: 4
31-56	5'-CCTCCGGTTCTGAAGGTGTTCTTGTA-3'	SEQ ID NO: 5
31-57	5'-GCCTCCGGTTCTGAAGGTGTTCTTGTA-3'	SEQ ID NO: 6
31-58	5'-TGCCTCCGGTTCTGAAGGTGTTCTTGTA-3'	SEQ ID NO: 7
32-53	5'-CCGGTTCTGAAGGTGTTCTTGT-3'	SEQ ID NO: 8
32-54	5'-TCCGGTTCTGAAGGTGTTCTTGT-3'	SEQ ID NO: 9
32-55	5'-CTCCGGTTCTGAAGGTGTTCTTGT 3'	SEQ ID NO: 10
32-56	5'-CCTCCGGTTCTGAAGGTGTTCTTGT-3'	SEQ ID NO: 11
32-57	5'-GCCTCCGGTTCTGAAGGTGTTCTTGT-3'	SEQ ID NO: 12
32-58	5'-TGCCTCCGGTTCTGAAGGTGTTCTTGT-3'	SEQ ID NO: 13
33-53	5'-CCGGTTCTGAAGGTGTTCTTG-3'	SEQ ID NO: 14
33-54	5'-TCCGGTTCTGAAGGTGTTCTTG-3'	SEQ ID NO: 15
33-55	5'-CTCCGGTTCTGAAGGTGTTCTTG-3'	SEQ ID NO: 16
33-56	5'-CCTCCGGTTCTGAAGGTGTTCTTG-3'	SEQ ID NO: 17
33-57	5'-GCCTCCGGTTCTGAAGGTGTTCTTG-3'	SEQ ID NO: 18
33-58	5'-TGCCTCCGGTTCTGAAGGTGTTCTTG-3'	SEQ ID NO: 19
34-53	5'-CCGGTTCTGAAGGTGTTCTT-3'	SEQ ID NO: 20
34-54	5'-TCCGGTTCTGAAGGTGTTCTT-3'	SEQ ID NO: 21
34-55	5'-CTCCGGTTCTGAAGGTGTTCTT-3'	SEQ ID NO: 22
34-56	5'-CCTCCGGTTCTGAAGGTGTTCTT-3'	SEQ ID NO: 23
34-57	5'-GCCTCCGGTTCTGAAGGTGTTCTT-3'	SEQ ID NO: 24
34-58	5'-TGCCTCCGGTTCTGAAGGTGTTCTT-3'	SEQ ID NO: 25
35-53	5'-CCGGTTCTGAAGGTGTTCT-3'	SEQ ID NO: 26
35-54	5'-TCCGGTTCTGAAGGTGTTCT-3'	SEQ ID NO: 27
35-55	5'-CTCCGGTTCTGAAGGTGTTCT-3'	SEQ ID NO: 28
35-56	5'-CCTCCGGTTCTGAAGGTGTTCT-3'	SEQ ID NO: 29
35-57	5'-GCCTCCGGTTCTGAAGGTGTTCT-3'	SEQ ID NO: 30
35-58	5'-TGCCTCCGGTTCTGAAGGTGTTCT-3'	SEQ ID NO: 31
36-53	5'-CCGGTTCTGAAGGTGTTC-3'	SEQ ID NO: 32
36-54	5'-TCCGGTTCTGAAGGTGTTC-3'	SEQ ID NO: 33
36-55	5'-CTCCGGTTCTGAAGGTGTTC-3'	SEQ ID NO: 34

36-56	5'-CCTCCGGTTCTGAAGGTGTTC-3'	SEQ ID NO: 35
36-57	5'-GCCTCCGGTTCTGAAGGTGTTC-3'	SEQ ID NO: 36
36-58	5'-TGCCTCCGGTTCTGAAGGTGTTC-3'	SEQ ID NO: 37

[0025]

An oligomer of the present invention preferably comprises a nucleotide sequence complementary to any one of the sequences consisting of nucleotides 32-56 (SEQ ID NO: 11), 33-56 (SEQ ID NO: 17), 34-56 (SEQ ID NO: 23), 35-56 (SEQ ID NO: 29) or 36-56 (SEQ ID NO: 35) from the 5' end of exon 53 of the human dystrophin gene.

Preferably, an oligomer of the present invention comprises a nucleotide sequence complementary to either of the sequences comprising nucleotides 32-56 (SEQ ID NO: 11) or 36-56 (SEQ ID NO: 35) from the 5' end of exon 53 in the human dystrophin gene.

[0026]

The term "enables skipping of exon 53 in the human dystrophin gene" means that, in the case of a DMD patient with deletion of exon 52 for example, by binding an oligomer of the present invention to a site corresponding to exon 53 of the transcript (for example, pre-mRNA) of the human dystrophin gene, when said transcript is spliced the nucleotide sequence corresponding to the 5' end of exon 54 is spliced to the 3′ side of the nucleotide sequence corresponding to the 3′ end of exon 51, so that no codon frame-shift occurs and mature mRNA is formed.

Therefore, as long as an oligomer of the present invention enables skipping of exon 53 of the dystrophin gene, it does not need to have a nucleotide sequence 100% complementary to the target sequence. For example, an oligomer of the present invention may include 1-3, 1-2, or 1 nucleotide non-complementary to the target sequence.

[0027]

In this connection, "binding" above means that when an oligomer of the present invention is mixed with a transcript of the human dystrophin gene, the two hybridize under physiological conditions to form double-stranded nucleic acid. Here, "under physiological conditions" means conditions of pH, salt composition and temperature adjusted to mimic the *in vivo*

environment. For example, conditions of 25-40°C, and preferably 37°C, pH 5-8, and preferably pH 7.4, and sodium chloride concentration 150 mM.

[0028]

It is possible to confirm whether skipping of exon 53 in the human dystrophin gene is produced by introducing the oligomer of the present invention into dystrophin-expressing cells (for example, human rhabdomyosarcoma cells), amplifying the region surrounding exon 53 of mRNA of the dystrophin gene from the total RNA of the dystrophin-expressing cells by RT-PCR and performing nested PCR or sequence analysis on said PCR-amplified product. By recovering human dystrophin gene mRNA from the test cells, measuring the quantity of polynucleotide "A" in the band for said mRNA in which exon 53 is skipped, and the quantity of polynucleotide "B" in the bands in which exon 53 has not been skipped, skipping efficiency can be calculated from the measured values for "A" and "B" by the following equation.

$$\text{Skipping efficiency (\%)} = A/(A + B) \times 100$$

[0029]

Oligomers of the present invention include oligomers in which nucleotides are the monomers, having a length of 18-28 nucleotides, with the nucleic acid monomers being linked by phosphate ester bonds, in other words oligonucleotides (hereinafter referred to as "oligonucleotides of the present invention"). These nucleotides can be either ribonucleotides or deoxyribonucleotide, and are preferably ribonucleotides. An oligonucleotide of the present invention can be synthesized easily by using different types of automated synthesizer (for example, AKTA oligopilot plus 10/100 (GE Healthcare)). Alternatively, the synthesis can also be entrusted to a third-party organization (for example, Promega Inc., or Takara Co.), etc.

[0030]

In addition, in order to heighten nuclease-resistance, etc., or stability in the body, an oligonucleotide of the present invention can have at least one modification of the

ribose or phosphate backbone constituting the nucleotides thereof. Such modifications include, for example, modifications of the ribose 2' position and modifications of other sites on the sugar, and modifications of the phosphate backbone. Modification of the ribose 2' position includes, for example, replacement of the 2'-OH of ribose by OR, R, R' OR, SH, SR, NH_2, NHR, NR_2, N_3, CN, F, Cl, Br or I. R here represents an alkyl or aryl. R as an alkyl is preferably a straight-chain or branched-chain C1-6 alkyl. Specific examples include methyl, ethyl, n-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, isopentyl, neopentyl, *tert*-pentyl, n-hexyl and isohexyl. This alkyl may optionally be substituted; examples of substituents here include halogens, alkoxy groups, cyano and nitro. There may be 1-3 of these substituents. The halogen(s) here can be fluorine, chlorine, bromine or iodine. The alkyl can be an alkyl mentioned above. The alkoxy can be a straight-chain or branched-chain C1-6 alkoxy, examples include methoxy, ethoxy, *n*-propoxy, isopropoxy, *n*-butoxy, isobutoxy, *sec*-butoxy, *tert*-butoxy, *n*-pentyloxy, isopentyloxy, *n*-hexyloxy and isohexyloxy, etc. A C1-3 alkoxy is particularly preferred. R as aryl is preferably a C6-10 aryl. Specific examples include phenyl, α-naphthyl and β-naphthyl. Phenyl is particularly preferred. R' represents an alkylene. R' as an alkylene is preferably a straight-chain or branched-chain C1-6 alkylene. Specific examples include methylene, ethylene, trimethylene, tetramethylene, pentamethylene, hexamethylene, 2-(ethyl)trimethylene and 1-(methyl)tetramethylene. The modifications of other sites on ribose include, for example, replacement of O at the 4' position by S, and constitution of artificial nucleic acid with a fixed configuration by bridging the 2' and 4' positions with $-O-CH_2-$. Examples of such artificial nucleic acids include LNA (locked nucleic acid) or ENA (2'-O,4'-C-ethylene-bridged nucleic acids), but are not limited to these.

[0031]

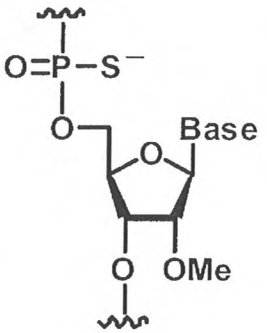
Modifications of the phosphate backbone includes, for example, modification by replacing the phosphodiester bond with a phosphorothioate bond, a phosphorodithioate bond, alkyl phosphonate bond, a phosphoroamidate bond or a boranophosphate

bond (Enya *et al.*: Bioorganic & Medicinal Chemistry, 2008, 18, 9154-9160) (see for example, Japan Domestic Re-Publications of WO2006/129594 A1 and WO2006/038608 A1).

[0032]

Preferably, an oligonucleotide of the present invention is an oligomer in which the monomer is -OMe-S-RNA, with the 2'-OH group of ribose replaced by -OMe (Me: methyl), and -O- in the phosphate group replaced by -S.

[Formula 4]



(In the formula, Me indicates methyl, and Base indicates any base or modified base of adenine, guanine, hypoxanthine, cytosine or uracil.)

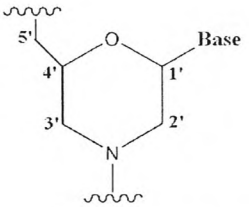
[0033]

Alternatively, an oligomer of the present invention can be an oligomer in which the monomers are nucleotide analogues. Nucleotide monomers include, for example, morpholino (compounds described in WO91/09033 A1) and peptide nucleic acids (PNA).

[0034]

A morpholino has the partial structure below. A morpholino differs from a nucleotide in that there is a morpholino ring rather than ribose. Methylene is bound to the 4' position on the morpholino ring. The 5' position is linked with a neighboring morpholino nitrogen atom via a group the main chain of which is constituted of 1-3 atoms.

[Formula 5]

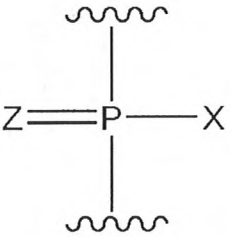


(In the formula, Base indicates adenine, guanine, hypoxanthine, cytosine, thymine or uracil, or a modified base.)

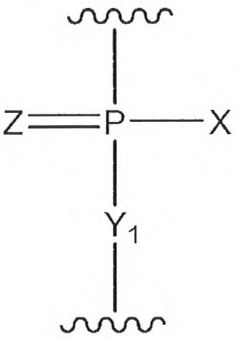
[0035]

The group, the main chain of which is constituted of 1-3 atoms, is represented by any of the formulae below.

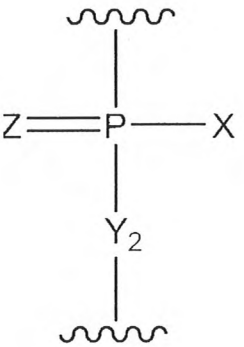
[Formula 6]



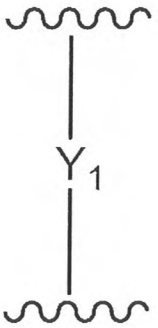
[Formula 7]



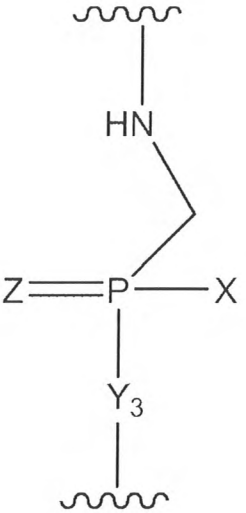
[Formula 8]



[Formula 9]



[Formula 10]



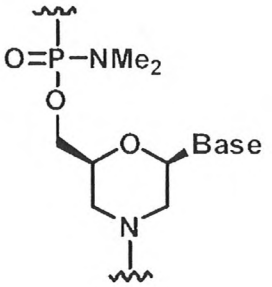
In the bound groups, X represents $-CH_2R^1$, $O-CH_2R^1$, $-S-CH_2R^1$, $-NR^2R^3$ or F; R^1 represents H or methyl, or an atom group which does not affect binding with the target sequence R^2 and R^3, can be mutually different, and are each R^1 or an alicyclic group or aromatic group; Y_1 is O, S, CH_2 or NR^1; Y_2 represents O, S or CH_2; Y_3 is O, S or NR^1 and Z is O or S.

For the details of morpholino structure see WO1992/009033 A1.

[0036]

In some embodiments, the oligomer of the present invention is a phosphorodiamidate represented by the formula below.

[Formula 11]



(In the formula, Me indicates methyl, and Base indicates adenine, guanine, hypoxanthine, cytosine, thymine or uracil, or a modified base.)

[0037]

Because the monomer in phosphorodiamidate morpholino oligomers (PMO) has the structure above, PMOs can form Watson-Crick base-pairs with other phosphorodiamidate morpholino chains or natural nucleotides (Corey, D. R. and Abrams, J. M. (2001) Genome Biol, 2, reviews 1015.1-1015.3). PMOs can maintain an antisense effect in cells for a long time, and because -O- in the phosphate group is replaced by NMe_2, PMOs are electrically neutral, and therefore, they have the advantage that they are not prone to non-specific binding with biomolecules other than the target gene. For processes for synthesizing PMOs see the following document: WP2009/064471.

[0038]

In an oligomer of the present invention the bases can be natural bases (adenine, guanine, hypoxanthine, cytosine, thymine or uracil), or they can be modified bases. Modified bases include, for example, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2,4,6-trimethoxybenzene, 3-methyluracil, dihydrouridine, naphthyl, aminophenyl, 5-alkycytidine (for example, 5-methylcytidine), 5-alkyuridine (for example, ribothymidine), 5-halouridine (5-bomouridine), 6-aza-pyrimidine, 6-alkylpyrimidine, (6-methyluridine), propene, queosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutoxosine, 4-acetyltidine, 5-(carboxyhydroxymethyl)uridine, 5-carboxymethylaminomethyl-2-thiouridine 5-carboxymethylamino-methyluridine, β-D-galactosylqueosine, 1-methyladenosine, 1-methylhypoxanthine, 2,2-dimethylguanosine, 3-methylcytosine, 2-methyladenosine, 2-methylguanosine, N6-methyladenosine,

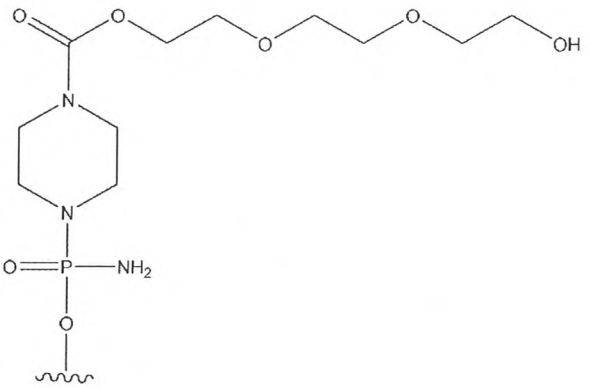
7-methylguanosine, 5-methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonylmethyluridine, 5-methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, β-D-galactmannosylqueosine, uridine-5-oxyacetic acid, 2-thiocytidine, threonine derivatives, purine, 2,6-diaminopurine, 2-diaminopurine, isoguanine, indole, imidazole, andxanthine, but are not limited to these.

[0039]

In addition, the 5'-methylene bound to ribose or the morpholino ring in the 5'-terminal nucleic acid residue of an oligomer of the present invention can be modified with any of the groups below.

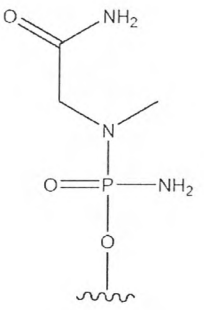
[Formula 12]

[Formula 13]



or

[Formula 14]



[0040]

2. Pharmaceutical composition

Oligomers of the present invention enable skipping of exon 53 with higher efficiency than antisense oligomers in the

prior art. Therefore, it is predicted that administration to DMD patients of a pharmaceutical composition which includes an oligomer of the present invention will enable highly efficient alleviation of the signs and symptoms of muscular dystrophy. For example, use of a pharmaceutical composition which includes an oligomer of the present invention is economic and can decrease adverse effects, because the same therapeutic effect can be achieved even with a smaller dose than with oligomers of the prior art.

Accordingly, as another embodiment, the present invention offers pharmaceutical compositions for treating muscular dystrophy, in which an oligomer of the present invention, a pharmaceutically permissible salt or a hydrate thereof is an active ingredient (hereinafter referred to as "compositions of the present invention").

[0041]

Pharmaceutically permissible salts of an oligomer included in a composition of the present invention include, for example, alkali metal salts such as sodium salts, potassium salts and lithium salts, alkaline earth metal salts such as calcium salts and magnesium salts, metal salts such as aluminum salts, iron salts, zinc salts, copper salts, nickel salts and cobalt salts, ammonium salts; organic amine salts such as *t*-octylamine salts, dibenzylamine salts, morpholine salts, glucosamine salts, phenylglycine alkyl ester salts, ethylenediamine salts, N-methylglucamine salts, guanidine salts, diethylamine salts, triethylamine salts, dicyclohexylamine salts, N,N-dibenzylethylenediamine salts, chloroprocaine salts, procaine salts, diethanolamine salts, N-benzylphenethylamine salts, piperazine salts, tetramethylammonium salts, tris(hydroxymethyl)aminomethane salts, hydrohalide salts such as hydrofluorates, hydrochlorides, hydrobromides and hydroiodides, inorganic acid salts such as nitrates, perchlorates, sulfates and phosphates, lower alkane sulfonates such as methanesulfonates, trifluoromethanesulfonates and ethanesulfonates, arylsulfonates such as benzenesulfonates and p-toluenesulfonates; organic acid salts such as acetates, malates, fumarates, succinates, citrates, tartrates, oxalates and maleates, and, amino acid salts such

as glycine salts, lysine salts, arginine salts, ornithine salts, glutamates and aspartates. These salts can be produced by a known process. Alternatively, oligomers of the present invention can be included in a composition of the present invention in the form of a hydrate thereof.

[0042]

There is no particular restriction as to the form of administration of a composition of the present invention, provided that it is a pharmaceutically permissible form of administration, and it can be selected to suit the method of treatment; however, from the point of view of ease of delivery to muscle tissues, intravenous administration, intra-arterial administration, intramuscular administration, subcutaneous administration, oral administration, intra-tissue administration or transdermal administration, etc., is preferred. There is also no particular restriction as to the dosage forms for a composition of the present invention, but examples include different types of injection, oral preparations, drips, inhalations, ointments and lotions.

[0043]

When an oligomer of the present invention is administered to a patient with muscular dystrophy, the composition of the present invention preferably includes a carrier which promotes delivery of the oligomer to muscle tissues. There is no particular restriction as to this carrier, provided that it is a pharmaceutically permissible carrier, but examples include cationic carriers such as cationic liposomes and cationic polymers, etc., or carriers using a viral envelope. Cationic liposomes include, for example, liposomes formed using 2-O-(2-diethylaminoethyl)carabamoyl-1,3-O-dioleoylglycerol and a phospholipid as essential constituents (hereinafter referred to as "liposome A"), Oligofectamine (registered trademark) (manufactured by Invitrogen Corp.), Lipofectin (registered trademark) (Invitrogen Corp.), Lipofectamine (registered trademark) (Invitrogen Corp.), Lipofectamine 2000 (registered trademark) (Invitrogen Corp.), DMRIE-C (registered trademark) (Invitrogen Corp.), GeneSilencer (registered trademark) (Gene Therapy Systems), TransMessenger (registered trademark) (QIAGEN, Inc.), TransIT TKO (registered trademark) (Mirus) and

Nucleofector II (Lonza). Of these, liposome A is preferred. Cationic polymers include, for example, JetSI (registered trademark) (Qbiogene, Inc.) and Jet-PEI (registered trademark) (polyethyleneimine, Qbiogene, Inc.). An example of a carrier using a viral envelope is GenomeOne (registered trademark) (HVJ-E liposome, Ishihara Sangyo). Alternatively, a pharmaceutical device described in JP 2924179 B2 and cationic carriers described in Japanese Domestic Re-Publication of WO2006/129594 A1 and WO2008/096690 A1 can also be used.

[0044]

The concentration of oligomer of the present invention included in a composition of the present invention will vary depending on the carrier, etc., but a concentration in the range of 0.1 nM to 100 μM is appropriate, with a range of 1 nM to 10 μM being preferred, and a range of 10 nM to 1 μM being more preferred. The ratio by weight of oligomer of the present invention and carrier contained in a composition of the present invention (carrier/oligomer of the present invention) will vary depending on the nature of the oligomer and the type of the carrier, etc., but a ratio in the range of 0.1-100 is appropriate, with a range of 1-50 being preferred, and a range of 10-20 being more preferred.

[0045]

In addition to an oligomer of the present invention and a carrier described above, a composition of the present invention can also optionally include pharmaceutically permissible additives. Such additives include, for example, emulsification aids (for example, C6-22 fatty acids and pharmaceutically permissible salts thereof, albumin and dextran), stabilizers (for example, cholesterol and phosphatidic acid), isotonic agents (for example, sodium chloride, glucose, maltose, lactose, sucrose and trehalose), and pH-regulating agents (for example, hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid, sodium hydroxide, potassium hydroxide and triethanolamine). One or more of these additives can be employed. As the content of these additives in a composition of the present invention ≤90 wt% is appropriate, and it is preferably ≤70 wt%, and more preferably ≤50 wt%.

[0046]

A composition of the present invention can be prepared by adding an oligomer of the present invention to a carrier dispersion and adequately stirring the mixture. Additives may also be added at an appropriate stage, before or after adding an oligomer of the present invention. There are no particular restrictions as to aqueous solvents that can be used when adding a double-stranded RNA oligo oligomer of the present invention, provided that it is pharmaceutically permissible, but examples include injectable water or injectable distilled water, electrolyte solutions such as physiological saline, and sugar solutions such as glucose solution or maltose solution. A person skilled in the art can select conditions such as pH and temperature for this, as appropriate.

[0047]

A composition of the present invention can be made into a liquid preparation or a lyophilized such preparation. A lyophilized preparation can be prepared by freeze-drying a liquid composition of the present invention by a conventional process. For example, after suitable sterilization, a composition of the present invention in the form of a liquid preparation can be dispensed in set quantities into vials, subjected to preliminary freezing for 2 hours at ca -40 to -20°C, and subjected to primary drying at ca 0-10°C under reduced pressure, and then lyophilized by secondary drying at ca 15-25°C under reduced pressure. In addition, the vials are generally sparged with nitrogen, and capped, to give a lyophilized preparation of a composition of the present invention.

[0048]

A lyophilized preparation of a composition of the present invention can be employed after redissolving, in general by adding a discretionary suitable solution (reconstituting liquid). The reconstituting liquid here can be injectable water, physiological saline or another common carrier liquid. There is no particular restriction as to the volume of the reconstituting liquid, which will vary depending on the intended use, etc., but 0.5-2 times the volume prior to lyophilization, or ≤500 mL, is appropriate.

[0049]

When administering a composition of the present invention, the dose is preferably adjusted, taking into account the type and dosage form of the oligomer of the present invention, the characteristics of the patient, such as age and bodyweight, the route of administration, and the nature and severity of the disease; however, the daily dose as the quantity of the oligomer of the present invention is generally in the range of 0.1 mg to 10 g/body, and preferably 1 mg to 1 g/body. These values may vary depending on type of target disease, route of administration and target molecule. Therefore, in some cases a dose lower than this range may be sufficient, and conversely, a dose higher than the range may sometimes be necessary. Administration can be once to several times daily, or at intervals of one to several days.

[0050]

As a separate embodiment of a composition of the present invention, a pharmaceutical composition which includes a vector capable of expressing an oligonucleotide of the present invention, and a carrier described above. This expression vector can be a vector capable of expressing a plurality of oligonucleotides of the present invention. As in the case of a composition of the present invention containing an oligomer of the present invention, such a composition can include pharmaceutically permissible additives. The concentration of expression vector included in the composition will vary depending upon the carrier, etc., but a concentration in the range of 0.1 nM to 100 μM is appropriate, a concentration in the range of 1 nM to 10 μM is preferred, and a concentration in the range of 10 nM to 1 μM is more preferred. The ratio by weight of the expression vector and the carrier contained in the composition (carrier/expression vector) will vary depending on the nature of the expression vector and the carrier, etc., but a ratio in the range of 0.1 to 100 is appropriate, a ratio in the range of 1 to 50 is preferred, and a ratio in the range of 10 to 20 is more preferred. The content of the carrier included in the composition is the same as in the case of a composition of the present invention containing an oligomer of the present invention; and the

method of preparation, etc. is also the same as for composition of the present invention.

[0051]

The present invention is described in more detail below citing practical examples thereof and experimental examples below; however, the present invention is not limited to the description in the examples.

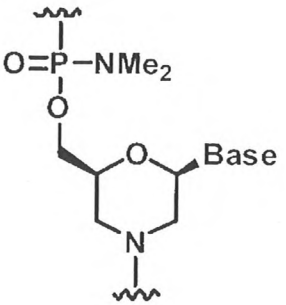
[Examples]

[0052]

[Synthesis Example 1]

The morpholino oligomers (PMO) shown in Table 2 below were synthesized as described in WO2009/064471 A1, by using an AKTA oligopilot plus 10 (GE Healthcare). The synthesized morpholino oligomers were dissolved in injectable water (Otsuka Pharmaceutical Factory, Inc.). The monomer in a morpholino oligomer is presented below.

[Formula 15]



(Me is methyl.)

[Table 2]

PMO No.	Target sequence in exon 53	Note	PMO Sequence
1	31-55	5′ end modifying group: -OH	SEQ ID NO: 4
2	32-53	5′ end modifying group: -OH	SEQ ID NO: 8
3	32-56	5′ end modifying group: -OH	SEQ ID NO: 11
4	33-54	5′ end modifying group: -OH	SEQ ID NO: 15
5	34-58	5′ end modifying group: -OH	SEQ ID NO: 25
6	36-55	5′ end modifying group: -OH	SEQ ID NO: 34
7	36-53	5′ end modifying group: -OH	SEQ ID NO: 32
8	36-55	5′ end modifying group: -OH	SEQ ID NO: 34

9	36-56	5′ end modifying group: -OH	SEQ ID NO: 35
10	36-57	5′ end modifying group: -OH	SEQ ID NO: 36
11	33-57	5′ end modifying group: -OH	SEQ ID NO: 18
12	39-69	5' end of sequence H53A(+39+69) in Non-Patent Document 3 (see Table 1)	SEQ ID NO: 38
13	30-59	5' end of sequence h53A30/1 in Non-Patent Document 5 (see Table 1)	SEQ ID NO: 39

[0053]

[Experimental Example 1]

In vitro assay

Antisense oligomers No. 1-9 and 12 were introduced at 10 μM into 4×10^5 RD cells (human rhabdomyosarcoma cell line) by means of a Nucleofector II (Lonza), using Amaxa Cell Line Nucleofector Kit L. The program used was T-030.

[0054]

After introduction, the cells were cultured overnight in 2 mL of Eagle's minimal essential medium (EMEM) (Sigma; the same applies below) containing 10% fetal calf serum (FCS) (Invitrogen) under conditions of 37°C and 5% CO_2. The cells were washed twice with PBS (Nissui; the same applies below), and then 500 μl of ISOGEN (Nippon Gene) was added to the cells; the cells were lysed by standing at room temperature for several minutes, and the lysate was recovered in Eppendorf tubes. Total RNA was extracted in accordance with the protocol included with ISOGEN. The concentration of the extracted total RNA was determined using a NanoDrop ND-1000 (LMS).

[0055]

One-Step RT-PCR was carried out on 400 ng of the extracted total RNA using a Titan One Tube RT-PCR Kit (Roche). Reaction solutions were prepared in accordance with the protocol included in the kit. A PTC-100 (MJ Research) was used as a thermal cycler. The RT-PCR program used was as follows.

50°C, 30 min: reverse transcription

94°C, 2 min: thermal denaturation

[94°C, 10 s; 58°C, 30 s; 68 °C, 45 s] × 30 cycles: PCR amplification
68°C, 7 min: thermal deactivation of the polymerase

[0056]

The nucleotide sequences of the forward primer and reverse primer used for RT-PCR are given below.

Forward primer: 5'-AGGATTTGGAACAGAGGCGTC-3' (SEQ ID NO: 40)
Reverse primer: 5'-GTCTGCCACTGGCGGAGGTC-3' (SEQ ID NO: 41)

[0057]

Next, nested PCR was carried out on the product amplified by RT-PCR above, using a Taq DNA Polymerase (Roche). The PCR program used was as follows.

94°C, 2 min: thermal denaturation
[94°C, 15 s; 58°C, 30 s; 68°C, 45 s] × 30 cycles: PCR amplification
68°C, 7 min: thermal deactivation of the polymerase

[0058]

The nucleotide sequences of the forward primer and reverse primer used for the nested PCR above are given below.

Forward primer: 5'-CATCAAGCAGAAGGCAACAA-3' (SEQ ID NO: 42)
Reverse primer: 5'-GAAGTTTCAGGGCCAAGTCA-3' (SEQ ID NO: 43)

[0059]

1 µl of the products of the nested PCR reaction above were analyzed using a Bioanalyzer (Agilent Technologies, Inc.).

The quantity of polynucleotide "A" in the band with skipping of exon 53 and the quantity of polynucleotide "B" in the band without skipping of exon 53 were measured. Skipping efficiency was found from the values measured for "A" and "B" by the following equation.

$$\text{Skipping efficiency (\%)} = A/(A+B) \times 100$$

[0060]

Experimental results

The results are shown in Figure 1. This experiment showed that antisense oligomers No. 1-9 all brought about skipping of exon 53 with a markedly higher efficiency than antisense oligomer No. 12. In particular, antisense oligomers No. 3 and 9 showed an exon skipping efficiency at least 4 times as high as antisense oligomer No. 12.

[0061]

[Experimental Example 2]

In vitro assay using human fibroblasts

Human myoD gene (SEQ ID NO: 44) was introduced into TIG-119 cells (fibroblasts from normal human tissue, National Institute of Biomedical Innovation) or 5017 cells (fibroblasts from a human DMD patient, Coriell Institute for Medical Research) by using ZsGreen1 coexpression retroviral vectors.

[0062]

After incubation for 4-5 days, MyoD-transformed fibroblasts ZsGreen-positive by FACS analysis were recovered and inoculated into a 12-well plate to give $5\times10^4/cm^2$. The growth medium employed was 1 mL of Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM F-12) (Invitrogen Corp.) including 10% FCS and 1% Penicillin/Streptomycin (P/S) (Sigma-Aldrich, Inc.).

[0063]

After 24 hours, the medium was replaced by differentiation medium (DMEM/F-12 containing 2% equine serum (Invitrogen Corp.), 1% P/S and ITS Liquid Media Supplement (Sigma, Inc.)). Incubation was continued for 12-14 days, exchanging the medium every 2-3 days, to bring about differentiation into myotube cells.

[0064]

After this, the differentiation medium was replaced by differentiation medium containing 6 μM Endo-Porter (Gene

Tools), and morpholino oligomers were added to give a final concentration of 10 μM. After incubation for 48 hours, total RNA was extracted from the cells by means of TRIzol (Invitrogen Corp.). RT-PCR was performed on 50 ng of the extracted total RNA using a QIAGEN OneStep RT-PCR Kit. The reaction solution was prepared in accordance with the accompanying protocol attached to the kit. An iCycler (Bio-Rad) was used as a thermal cycler. The RT-PCR program used was as follows.

50°C, 30 min: reverse transcription
95°C, 15 min: thermal denaturation
[94°C, 1 min; 60°C, 1 min; 72°C, 1 min] × 35 cycles: PCR amplification
72°C, 7 min: thermal deactivation of the polymerase

[0065]
The primers employed were hEX51F and hEX55R.

hEX51F: 5'-CGGGCTTGGACAGAACTTAC-3' (SEQ ID NO: 45)
hEx55R: 5'-TCCTTACGGGTAGCATCCTG-3' (SEQ ID NO: 46)

[0066]
The products of the RT-PCR reaction above were separated by 2% agarose gel electrophoresis, and gel images were captured using GeneFlash (Syngene). The quantity of polynucleotide "A" in the band with skipping of exon 53 and the quantity of polynucleotide "B" in the band without skipping of exon 53 were measured using ImageJ (National Institutes of Health, USA). Skipping efficiency was found from the values measured for "A" and "B" by the following equation.

$$\text{Skipping efficiency (\%)} = A/(A+B) \times 100$$

[0067]
Experimental results
The results are shown in Figure 2 and 3. This experiment showed that in TIG-119 cells, antisense oligomers No. 3, 9 and 10 (Figure 2) all brought about skipping of exon 53 with

higher efficiency than antisense oligomer No. 13 (Figure 2). In particular, antisense oligomers No. 3 and 9 showed exon skipping efficiency at least twice as high as that of antisense oligomer No. 13 (Figure 2).

This experiment also showed that, in 5017 cells, antisense oligomers No. 3 and 9-11 (Figure 3) all brought about skipping of exon 53 with a higher efficiency than antisense oligomer No. 13 (Figure 3). In particular, antisense oligomers No. 3 and 9 showed exon skipping efficiency at least seven times as high as that of antisense oligomer No. 13 (Figure 3).

[Industrial Applicability]

[0068]

The experimental results presented in the experimental examples indicate that, in all the cellular environments, oligomers of the present invention (No. 1-11) brought about skipping of exon 53 with markedly higher efficiency than oligomers of the prior art (No. 12 and 13). The 5017 cells used in Experimental Example 2 were cells collected from a patient with Duchenne muscular dystrophy; and therefore, oligomers of the present invention can also be expected to bring about highly efficient skipping of exon 53 when actually administered to patients with Duchenne muscular dystrophy.

Therefore, the oligomers of the present invention are very useful for the treatment of Duchenne muscular dystrophy.

[Sequence listing free text]

[0069]

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Sequence listing:

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NATIONAL CENTER OF NEUROLOGY AND PSYCHIATRY

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[Document] Claims

[Claim 1]

Antisense oligomer which is an antisense oligomer which enables skipping of exon 53 of the human dystrophin gene, comprising a nucleotide sequence complementary to any one of the sequences comprising nucleotides 31-53, 31-54, 31-55, 31-56, 31-57, 31-58, 32-53, 32-54, 32-55, 32-56, 32-57, 32-58, 33-53, 33-54, 33-55, 33-56, 33-57, 33-58, 34-53, 34-54, 34-55, 34-56, 34-57, 34-58, 35-53, 35-54, 35-55, 35-56, 35-57, 35-58, 36-53, 36-54, 36-55, 36-56, 36-57 or 36-58 from the 5' end of exon 53 of the human dystrophin gene.

[Claim 2]

Antisense oligomer according to claim 1, which is an oligonucleotide.

[Claim 3]

Antisense oligomer according to claim 2, wherein a sugar moiety and/or phosphate constituting the aforementioned oligonucleotide is modified.

[Claim 4]

Antisense oligomer according to claim 3, wherein a sugar moiety constituting the aforementioned oligonucleotide is ribose in which the 2′-OH group is replaced by any group selected from a set comprising OR, R, R′ OR, SH, SR, NH_2, NHR, NR_2, N_3, CN, F, Cl, Br and I.
(Where R indicates a C1-6 alkyl or C1-6 aryl and R′ indicates a C1-6 alkylene.)

[Claim 5]

Antisense oligomer according to claim 3 or 4, wherein a phosphate bond constituting the oligonucleotide is any one selected from a set comprising a phosphorothioate bond, a phosphorodithioate bond, an alkylphosphonate bond and a phosphoroamidate bond.

[Claim 6]

Antisense oligomer according to claim 1, which is a morpholino oligomer.

[Claim 7]

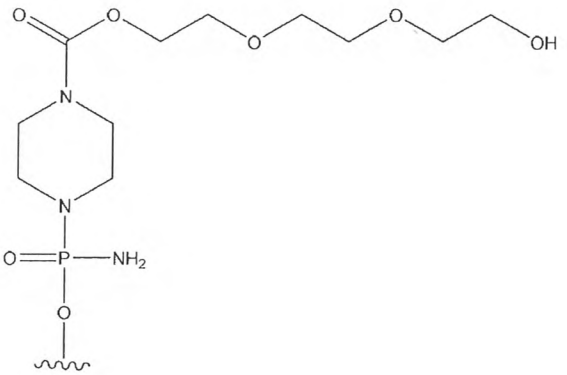
Antisense oligomer according to claim 6, which is a phosphoroamidate morpholino oligomer.

[Claim 8]

Antisense oligomer according to any one of claims 1-7, wherein the 5' methylene bound to ribose or the morpholino ring of the 5' terminal nucleic acid residue is modified with any one of the groups below:

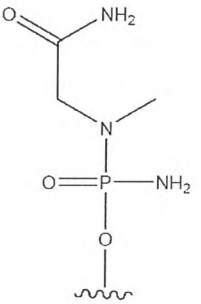
[Formula 16]

[Formula 17]



or

[Formula 18]



[Claim 9]

Antisense oligomer according to any one of claims 1-8, comprising a nucleotide sequence complementary to a sequence comprising nucleotides 32-56 or 36-56 from the 5' end of exon 53 of the human dystrophin gene.

[Claim 10]

Antisense oligomer according to any one of claims 1-8, comprising any one nucleotide sequence selected from a set comprising SEQ ID NO: 2-37.

[Claim 11]

Antisense oligomer according to any one of claims 1-8, comprising any one nucleotide sequence selected from a set comprising SEQ ID NO: 11, 17, 23, 29 and 35.

[Claim 12]

Antisense oligomer according to any one of claims 1-8, comprising the nucleotide sequence of either SEQ ID NO: 11 or 35.

[Claim 13]

Pharmaceutical composition for treating muscular dystrophy, in which an active ingredient is an antisense oligomer according to any one of claims 1 to 12, or a pharmaceutically permissible salt or hydrate thereof.

[Document] Abstract

[Abstract]

[Problem] To offer drugs which bring about skipping of exon 53 of the human dystrophin gene with high efficiency.

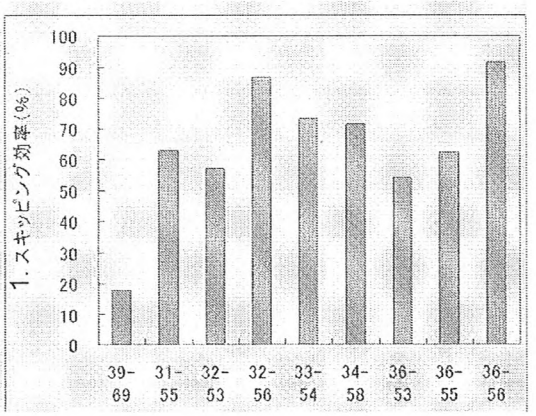
[Solution] The present invention offers oligomers which enable skipping of exon 53 of the human dystrophin gene.

[Selected Drawing] None

[Document] Drawings

[Figure 1]

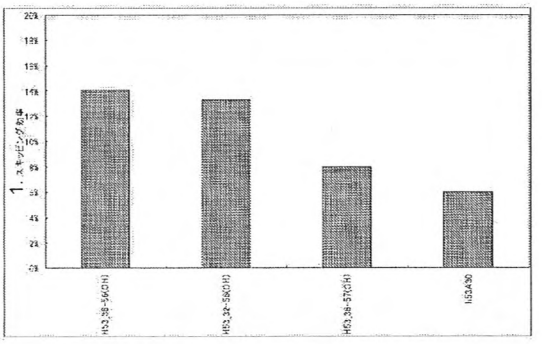
Figure 1. Investigation in RD



1. Skipping efficiency (%)

[Figure 2]

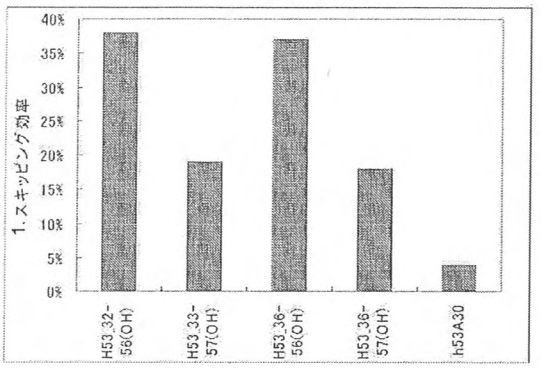
Figure 2. Investigation in TIG-119



1. Skipping efficiency (%)

[Figure 3]

Figure 3 Investigation in 5017



1. Skipping efficiency (%)

APPLICANTS

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New Registration

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NIPPON SHINYAKU CO., LTD.
510147776
20100527
New Registration

4-1-1 Ogawa-Higashi, Kodaira, Tokyo-to
NATIONAL CENTER OF NEUROLOGY AND PSYCHIATRY

UNITED STATES PATENT AND TRADEMARK OFFICE

I, Brian William HOWELLS,

translator to RWS Group Ltd, of Europa House, Chiltern Park, Chiltern Hill, Chalfont St Peter, Buckinghamshire, United Kingdom, declare;

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland.

2. That I am well acquainted with the Japanese and English languages.

3. That the attached is, to the best of my knowledge and belief, a true translation into the English language of the accompanying copy of the specification filed with the application for a patent in Japan on September 1, 2010 under the number JP-2010-196032 and the official certificate attached thereto.

4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application in the United States of America or any patent issuing thereon.

Date: November 22, 2019

B. W. HOWELLS

For and on behalf of RWS Group Ltd

EXHIBIT AI

8145738

THE UNITED STATES OF AMERICA

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United States Patent and Trademark Office

August 12, 2021

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APPLICATION NUMBER: *14/615,504*
FILING DATE: *February 06, 2015*
PATENT NUMBER: *9708361*
ISSUE DATE: *July 18, 2017*

Certified by

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PTO/AIA/15 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

UTILITY PATENT APPLICATION TRANSMITTAL

(ONLY FOR NEW NONPROVISIONAL APPLICATIONS UNDER 37 CFR 1.53(B))

Attorney Docket No.	209658-0001-01-US-518587
First Named Inventor	Naoki WATANABE
Title	ANTISENSE NUCLEIC ACIDS
Express Mail Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [X] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [ ] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [X] **Specification** [*Total Pages* 66]
   Both the claims and abstract must start on a new page.
   (See MPEP § 608.01(a) for information on the preferred arrangement)
5. [X] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 19]
6. **Inventor's Oath or Declaration** [*Total Pages* 4]
   (including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))
   a. [X] Newly executed (original or copy)
   b. [ ] A copy from a prior application (37 CFR 1.63(d))
7. [X] **Application Data Sheet** * *See note below.*
   See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R**
   in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission**
   (if applicable, items a. – c. are required)
   a. [X] Computer Readable Form (CRF)
   b. [X] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [X] Paper
   c. [X] Statements verifying identity of above copies

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ACCOMPANYING APPLICATION PAPERS

10. [ ] **Assignment Papers**
    (cover sheet & document(s))
    Name of Assignee
11. [ ] **37 CFR 3.73(c) Statement** (*when there is an assignee*) [ ] **Power of Attorney**
12. [ ] **English Translation Document** (*if applicable*)
13. [X] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
14. [X] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [ ] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [X] **Other:** Request to Retrieve Electronic Copy of Priority Applications

*Note: (1) Benefit claims under 37 CFR 1.76 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

[X] The address associated with Customer Number: 055694 OR [ ] Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature		Date	February 6, 2015
Name (Print/Type)	Zhengyu Feng, Ph.D.	Registration No. (Attorney/Agent)	66,816

78644591.1

PTO/AIA/01 (06-12)
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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	ANTISENSE NUCLEIC ACIDS

As the below named inventor, I hereby declare that:

This declaration is directed to:
- [x] The attached application, or
- [ ] United States application or PCT international application number _____ filed on _____.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Naoki WATANABE Date (Optional): Nov. 26, 2014

Signature: Naoki Watanabe

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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LEGAL NAME OF INVENTOR

Inventor: Youhei SATOU Date (Optional): Nov. 21, 2014

Signature: Youhei Satou

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LEGAL NAME OF INVENTOR

Inventor: Shin'ichi TAKEDA Date (Optional): 21 Nov, 2014

Signature: Shin'ichi Takeda

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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This declaration is directed to:
- [x] The attached application, or
- [ ] United States application or PCT international application number _____ filed on _____.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

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LEGAL NAME OF INVENTOR

Inventor: Tetsuya NAGATA Date (Optional): 25 Nov, 2014

Signature: Tetsuya Nagata

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	209658-0001-01-US-518587
	Application Number	
Title of Invention	ANTISENSE NUCLEIC ACIDS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Naoki		WATANABE	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

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Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Youhei		SATOU	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

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Mailing Address of Inventor:

Address 1	Room 402, Ruvio II, 21-3, Sakura 1-chome		
Address 2			
City	Tsukuba-shi, Ibaraki	State/Province	
Postal Code	305-0003	Country [i]	JP

Inventor 3 Remove

Legal Name

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	209658-0001-01-US-518587
	Application Number	
Title of Invention	ANTISENSE NUCLEIC ACIDS	

Prefix	Given Name	Middle Name	Family Name	Suffix
	Shin'ichi		TAKEDA	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

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Address 2	1-1, Ogawahigashicho 4-chome		
City	Kodaira-shi, Tokyo	**State/Province**	
Postal Code	187-8551	**Country** i	JP

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Tetsuya		NAGATA	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Kodaira-shi, Tokyo	Country of Residence i	JP

Mailing Address of Inventor:

Address 1	c/o National Center of Neurology and Psychiatry		
Address 2	1-1, Ogawahigashicho 4-chome		
City	Kodaira-shi, Tokyo	**State/Province**	
Postal Code	187-8551	**Country** i	JP

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	055694		
Email Address	DBRIPDocket@dbr.com	Add Email	Remove Email

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	209658-0001-01-US-518587
	Application Number	
Title of Invention	ANTISENSE NUCLEIC ACIDS	

Application Information:

Title of the Invention	ANTISENSE NUCLEIC ACIDS		
Attorney Docket Number	209658-0001-01-US-518587	**Small Entity Status Claimed** ☐	
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	19	**Suggested Figure for Publication (if any)**	

Filing By Reference :

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country i

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	055694		

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	209658-0001-01-US-518587
	Application Number	
Title of Invention	ANTISENSE NUCLEIC ACIDS	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	13891520	2013-04-10
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13891520	a 371 of international	PCT/JP2011/070318	2011-08-31

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) [i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country [i]	Filing Date (YYYY-MM-DD)	Access Code [i] (if applicable)
2010-196032	JP	2010-09-01	

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	209658-0001-01-US-518587
	Application Number	
Title of Invention	ANTISENSE NUCLEIC ACIDS	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	209658-0001-01-US-518587
	Application Number	
Title of Invention	ANTISENSE NUCLEIC ACIDS	

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	NIPPON SHINYAKU CO., LTD.

Mailing Address Information For Applicant:

Address 1	14, Kisshoin Nishinosho Monguchicho, Minami-ku		
Address 2			
City	Kyoto-shi, Kyoto	**State/Province**	
Country [i]	JP	Postal Code	601-8550
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Applicant 2 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

PTO/AIA/14 (12-13)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	209658-0001-01-US-518587
	Application Number	
Title of Invention	ANTISENSE NUCLEIC ACIDS	

If the Applicant is an Organization check here. ☒			
Organization Name	NATIONAL CENTER OF NEUROLOGY AND PSYCHIATRY		
Mailing Address Information For Applicant:			
Address 1	1-1, Ogawahigashicho 4-chome		
Address 2			
City	Kodaira-shi, Tokyo	**State/Province**	
Country i JP		Postal Code	187-8551
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☒			
Organization Name	NIPPON SHINYAKU CO., LTD.		
Mailing Address Information For Assignee including Non-Applicant Assignee:			
Address 1	14, Kisshoin Nishinosho Monguchicho, Minami-ku		
Address 2			
City	Kyoto-shi, Kyoto	**State/Province**	
Country i JP		Postal Code	601-8550
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

PTO/AIA/14 (12-13)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	209658-0001-01-US-518587
	Application Number	
Title of Invention	ANTISENSE NUCLEIC ACIDS	

Assignee 2

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☒

Organization Name	NATIONAL CENTER OF NEUROLOGY AND PSYCHIATRY

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1	1-1, Ogawahigashicho 4-chome		
Address 2			
City	Kodaira-shi, Tokyo	**State/Province**	
Country i	JP	Postal Code	187-8551
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Signature	/Zhengyu Feng/			Date (YYYY-MM-DD)	2015-02-06
First Name	Zhengyu	Last Name	Feng	Registration Number	66816

Additional Signature may be generated within this form by selecting the Add button. Add

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent C o o p eration Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Docket No.: 209658-0001-01-US-518587
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Naoki WATANABE et al.

Application No.: Not Yet Assigned | Confirmation No.: N/A

Filed: Concurrently Herewith | Art Unit: N/A

For: ANTISENSE NUCLEIC ACIDS | Examiner: Not Yet Assigned

FIRST PRELIMINARY AMENDMENT

MS NEW
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the Specification begin on page 2.

Amendments to the Claims begin on page 3.

Remarks begin on page 4.

AMENDMENTS TO THE SPECIFICATION

Please insert the following paragraph on page 1 of the specification before the section entitled "TECHNICAL FIELD":

CROSS REFERENCE TO RELATED APPLICATIONS

This is a Continuation of copending Application No. 13/819,520, filed April 10, 2013, which is a PCT National Stage of PCT/JP2011/070318 filed August 31, 2011, which claims priority to JP Application No. 2010-196032 filed September 1, 2010.

SEQUENCE LISTING

A Sequence Listing containing SEQ ID NO: 1-123 is incorporated herein by reference.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of the claims in the application:

LISTING OF CLAIMS

Claim 1. (Previously Amended): An antisense oligomer which causes skipping of the 53rd exon in the human dystrophin gene, consisting of a nucleotide sequence complementary to any one of the sequences consisting of the 32nd to the 56th or the 36th to the 56th nucleotides from the 5' end of the 53rd exon in the human dystrophin gene.

Claims 2-14. (Canceled).

REMARKS

This Preliminary Amendment is being filed concurrently with a continuation application. No prohibited new matter is believed to be added. Entry of this Preliminary Amendment is respectfully requested.

Applicants amend the Specification to (1) update the References to Related Application, and (2) refer to the Sequence Listing. The present Preliminary Amendment starts from the claims of PCT/JP2011/070318 as amended under Article 34 on June 20, 2012 (a copy and the English translation thereof are enclosed). Applicants cancel claims 2-14. Applicants reserve the right to file a Continuation or Divisional application on the subject matter cancelled by way of this amendment.

CONCLUSION

If there are any other fees due in connection with the filing of this preliminary amendment, please charge the fees to our Deposit Account No. 50-0573. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Dated: February 6, 2015

Respectfully submitted,

By _____

Zhengyu Feng, Ph.D.
Registration No.: 66,816
DRINKER BIDDLE & REATH LLP
1500 K Street, N.W.
Suite 1100
Washington, DC 20005-1209
202.230.5119 (Phone)
202.842.8465 (Fax)
Attorneys/Agents For Applicant

Electronic Acknowledgement Receipt

EFS ID:	21419384
Application Number:	14615504
International Application Number:	
Confirmation Number:	2704
Title of Invention:	ANTISENSE NUCLEIC ACIDS
First Named Inventor/Applicant Name:	Naoki WATANABE
Customer Number:	55694
Filer:	Zhengyu Feng/Moira Kelly
Filer Authorized By:	Zhengyu Feng
Attorney Docket Number:	209658-0001-01-US-518587
Receipt Date:	06-FEB-2015
Filing Date:	
Time Stamp:	10:13:07
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1600
RAM confirmation Number	8506
Deposit Account	500573
Authorized User	FENG, ZHENGYU
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Fee Worksheet (SB06)	209658_0001_01_518587_Fee_Transmittal.pdf	97369 614b9181173bf60076e1846a4a249ac42e55bd8f	no	1
Warnings:					
Information:					
2	Transmittal of New Application	209658_0001_01_518587_Application_Transmittal.pdf	103899 1e40f853327513286bace56c363a3a82c1f2bed6	no	1
Warnings:					
Information:					
3		209658_0001_01_518587_Application.pdf	3713088 3afef0108b19d93c5aa99ab1f3a87d9557f2e5b7	yes	85

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	63
Claims	64	65
Abstract	66	66
Drawings-only black and white line drawings	67	85

Warnings:					
Information:					
4	Oath or Declaration filed	209658_0001_01_518587_Inventors_Declarations.pdf	264887 0d628280911f840656eb5426929f767341265590	no	4
Warnings:					
Information:					
5	Application Data Sheet	209658_0001_01_518587_Application_Data_Sheet.PDF	1895463 4031f2d6b41558533f8a9e10c8a6e61f3278aa74	no	9
Warnings:					
Information:					

6	Request for Transfer of a Computer Readable Form	209658_0001_01_518587_Request_Transfer_Sequence_Listing.pdf	96131 f1da247a55d0a5a02bbb0c1b63d78296a246f95d	no	1
Warnings:					
Information:					
7	Preliminary Amendment	209658_0001_01_518587_First_Preliminary_Amendment.pdf	297578 104128c15f6210c93e91a8d97e6cd1b69b7fb552	no	9
Warnings:					
Information:					
8		209658_0001_01_518587_IDS.pdf	244363 34dacce661912e19e42a825df5b64c258ee381fe	yes	4
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Transmittal Letter		1	2	
	Information Disclosure Statement (IDS) Form (SB08)		3	4	
Warnings:					
Information:					
9	Request for USPTO to retrieve priority docs	209658_0001_01_518587_Request_Retrieve_Priority_Application.pdf	106676 7e1dc55b73cc5fce30475af2c1fb9496f89ef018	no	1
Warnings:					
Information:					
10	Sequence Listing (Text File)	209658_0001_01_518587_Sequence_Listing.TXT	24635	no	0
Warnings:					
Information:					
11	Fee Worksheet (SB06)	fee-info.pdf	35013 a5c03106ca581f82e47df68af4028248b84126ed	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	6879102		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Docket No.: 209658-0001-01-US-518587
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Naoki WATANABE et al.

Application No.: 14/615,504 Confirmation No.: 2704

Filed: February 6, 2015 Art Unit: N/A

For: ANTISENSE NUCLEIC ACIDS Examiner: Not Yet Assigned

SECOND PRELIMINARY AMENDMENT

MS AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims begin on page 2.

Remarks begin on page 5.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of the claims in the application:

LISTING OF CLAIMS

Claim 1. (Currently Amended): An antisense oligomer which causes skipping of the 53rd exon in the human dystrophin gene, consisting of a nucleotide sequence complementary to any one of the sequences consisting of: the 31st to the 55th, the 32nd to the 53rd, the 32nd to the 56th, the 32nd to the 61st, the 33rd to the 54th, the 33rd to the 57th, the 34th to the 58th, the 35th to the 59th, the 36th to the 53rd, the 36th to the 55th, the 36th to the 57th, the 36th to the 60th, or the 37th to the 61st ~~or the 36th to the 56th~~ nucleotides from the 5' end of the 53rd exon in the human dystrophin gene.

Claims 2-14. (Canceled).

Claim 15. (New): The antisense oligomer according to claim 1, which is an oligonucleotide.

Claim 16. (New): The antisense oligomer according to claim 15, wherein the sugar moiety and/or the phosphate-binding region of at least one nucleotide constituting the oligonucleotide is modified.

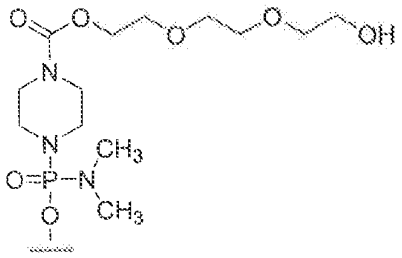
Claim 17. (New): The antisense oligomer according to claim 16, wherein the sugar moiety of at least one nucleotide constituting the oligonucleotide is a ribose in which the 2'-OH group is replaced by any one selected from the group consisting of: OR, R, R'OR, SH, SR, NH_2, NHR, NR_2, N_3, CN, F, Cl, Br, and I, wherein R is an alkyl or an aryl and R' is an alkylene.

Claim 18. (New): The antisense oligomer according to claim 16, wherein the phosphate-binding region of at least one nucleotide constituting the oligonucleotide is any one selected from the group consisting of: a phosphorothioate bond, a phosphorodithioate bond, an alkylphosphonate bond, a phosphoramidate bond, and a boranophosphate bond.

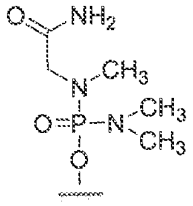
Claim 19. (New): The antisense oligomer according to claim 1, which is a morpholino oligomer.

Claim 20. (New): The antisense oligomer according to claim 19, which is a phosphorodiamidate morpholino oligomer.

Claim 21. (New): The antisense oligomer according to claim 19, wherein the 5' end is any one of the groups of chemical formulae (1) to (3) below:



(1)



(2)

(3)

Claim 22. (New): The antisense oligomer according to claim 1, consisting of a nucleotide sequence complementary to the sequences consisting of the 32nd to the 56th nucleotides from the 5' end of the 53rd exon in the human dystrophin gene.

Claim 23. (New): The antisense oligomer according to claim 1, consisting of the nucleotide sequence shown by any one selected from the group consisting of SEQ ID NOS: 4, 8, 11, 15, 18, 25, 32, 34, 36, 57, 103, 104, 105, and 109.

Claim 24. (New): The antisense oligomer according to claim 1, consisting of the nucleotide sequence shown by SEQ ID NO: 11.

Claim 25. (New): A pharmaceutical composition for the treatment of muscular dystrophy, comprising as an active ingredient the antisense oligomer according to claim 1, or a pharmaceutically acceptable salt or hydrate thereof.

REMARKS

Applicants herewith amend claim 1 and add new claims 15-25. Support for the amendments can be found at least from (1) the original claims of the PCT application, and (2) TEST EXAMPLE 7 and Table 7 of the Specification. No prohibited new matter is believed to be added. Applicants reserve the right to file a divisional or continuation application on any subject matter canceled by amendment. The cancelation of subject is without prejudice to, or disclaimer of, the subject matter.

CONCLUSION

If there are any other fees due in connection with the filing of this preliminary amendment, please charge the fees to our Deposit Account No. 50-0573. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Dated: February 9, 2015

Respectfully submitted,

By_____

Zhengyu Feng, Ph.D.
Registration No.: 66,816
DRINKER BIDDLE & REATH LLP
1500 K Street, N.W.
Suite 1100
Washington, DC 20005-1209
202.230.5119 (Phone)
202.842.8465 (Fax)
Attorneys/Agents For Applicant

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82A (07-13)
Approved for use through 11/30/2014. OMB 0651-0051
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.

Application Number	14/615,504
Filing Date	February 6, 2015
First Named Inventor	Naoki WATANABE
Title	ANTISENSE NUCLEIC ACIDS
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	209658-0001-01-US-518587

SIGNATURE of Applicant or Patent Practitioner

Signature		Date (Optional)	February 9, 2015
Name	Zhengyu Feng, Ph.D.	Registration Number	66,816
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)			

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ *Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

POWER OF ATTORNEY

Each party signing this document hereby submits the following:

I/We am/are an Applicant in the application:

☐ identified below.

☒ identified in the attached transmittal letter (PTO/AIA/82A or equivalent).

(Note: check first box if Power of Attorney is specific to a single application; check second box if general Power of Attorney usable for multiple applications.)

I/We hereby revoke all previous powers of attorney in the identified application.

I/We hereby appoint Practitioners associated with Customer Number 055694 as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the identified application.

Please recognize or change the correspondence address for the identified application to the address associated with the above-identified Customer Number.

Identified Application Information (to the extent applicable/available):

Application No.:	
Filing Date:	
First Named Inventor:	
Title:	
Art Unit:	
Examiner Name:	
Attorney Docket Number:	

Note: If this section is left blank, then the instant Power of Attorney is for the application identified in any attached transmittal letter (PTO/AIA/82A or equivalent).

SIGNATURE of Assignee/Applicant for Patent

Signature	T Higuchi	Date	June 2, 2014
Name	Teruhiko HIGUCHI	Title	President
Company Name and Address	NATIONAL CENTER OF NEUROLOGY AND PSYCHIATRY 1-1, Ogawahigashi-cho 4-chome, Kodaira-shi, Tokyo 187-8551 Japan		

NOTE: Signature - this form must be signed by the Applicant or Applicant's representative in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. A statement under 37 CFR 3.73(c) should be filed along with a copy of this form in the case where the identified application has more than one assignee. The statement under 37 CFR 3.73(c) may be completed by one of the practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

ACTIVE/ 75636306.1

PTO/AIA/96 (08-12)
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STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: NATIONAL CENTER FOR NEUROLOGY AND PSYCHIATRY

Application No./Patent No.: 14/615,504 Filed/Issue Date: February 6, 2015

Titled: ANTISENSE NUCLEIC ACIDS

NATIONAL CENTER FOR NEUROLOGY AND PSYCHIATRY (Name of Assignee), a corporation (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☐ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

   ☐ The extent (by percentage) of its ownership interest is _____ %). Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☒ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   NIPPON SHINYAKU CO., LTD.

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 030185, Frame 0302, or for which a copy thereof is attached.

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

   1. From: _____ To: _____
      The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

   2. From: _____ To: _____
      The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

78414674.1

PTO/AIA/96 (08-12)
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STATEMENT UNDER 37 CFR 3.73(c)

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5. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Signature	February 9, 2015 Date
Zhengyu Feng, Ph.D. Printed or Typed Name	66,816 Title or Registration Number

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82A (07-13)
Approved for use through 11/30/2014. OMB 0651-0051
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.

Application Number	14/615,504
Filing Date	February 6, 2015
First Named Inventor	Naoki WATANABE
Title	ANTISENSE NUCLEIC ACIDS
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	209658-0001-01-US-518587

SIGNATURE of Applicant or Patent Practitioner

Signature		Date (Optional)	February 9, 2015
Name	Zhengyu Feng, Ph.D.	Registration Number	66,816
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)			

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ *Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

POWER OF ATTORNEY

Each party signing this document hereby submits the following:

I/We am/are an Applicant in the application:

☐ identified below.

☒ identified in the attached transmittal letter (PTO/AIA/82A or equivalent).

(Note: check first box if Power of Attorney is specific to a single application; check second box if general Power of Attorney usable for multiple applications.)

I/We hereby revoke all previous powers of attorney in the identified application.

I/We hereby appoint Practitioners associated with Customer Number 055694 as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the identified application.

Please recognize or change the correspondence address for the identified application to the address associated with the above-identified Customer Number.

Identified Application Information (to the extent applicable/available):

Application No.:	
Filing Date:	
First Named Inventor:	
Title:	
Art Unit:	
Examiner Name:	
Attorney Docket Number:	

Note: If this section is left blank, then the instant Power of Attorney is for the application identified in any attached transmittal letter (PTO/AIA/82A or equivalent).

SIGNATURE of Assignee/Applicant for Patent

Signature	Yoshiaki Shirouchi	Date	June 11, 2014
Name	Yoshiaki SHIROUCHI	Title	General Manager
Company Name and Address	NIPPON SHINYAKU CO., LTD. 14, Kisshoin Nishinosho Monguchicho, Minami-ku, Kyoto-shi, Kyoto 601-8550 Japan		

NOTE: Signature - this form must be signed by the Applicant or Applicant's representative in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. A statement under 37 CFR 3.73(c) should be filed along with a copy of this form in the case where the identified application has more than one assignee. The statement under 37 CFR 3.73(c) may be completed by one of the practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

ACTIVE/ 75636301.1

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: NIPPON SHINYAKU CO., LTD.

Application No./Patent No.: 14/615,504 Filed/Issue Date: February 6, 2015

Titled: ANTISENSE NUCLEIC ACIDS

NIPPON SHINYAKU CO., LTD. (Name of Assignee), a corporation (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose one of options 1, 2, 3 or 4 below):

1. ☐ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

☐ The extent (by percentage) of its ownership interest is _____ %). Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☒ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

NATIONAL CENTER FOR NEUROLOGY AND PSYCHIATRY

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose one of options A or B below):

A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 030185, Frame 0302, or for which a copy thereof is attached.

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

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The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

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The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

PTO/AIA/96 (08-12)
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STATEMENT UNDER 37 CFR 3.73(c)

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The document was recorded in the United States Patent and Trademark Office at
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5. From: _____ To: _____
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Reel _____, Frame _____, or for which a copy thereof is attached.

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The document was recorded in the United States Patent and Trademark Office at
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☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Signature	February 9, 2015 Date
Zhengyu Feng, Ph.D. Printed or Typed Name	66,816 Title or Registration Number

78414646.1

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/615,504	02/06/2015	Naoki WATANABE	209658-0001-01-US-518587	2704

55694 7590 03/25/2016

DRINKER BIDDLE & REATH (DC)
1500 K STREET, N.W.
SUITE 1100
WASHINGTON, DC 20005-1209

EXAMINER	
MCGARRY, SEAN	

ART UNIT	PAPER NUMBER
1674	

NOTIFICATION DATE	DELIVERY MODE
03/25/2016	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DBRIPDocket@dbr.com
penelope.mongelluzzo@dbr.com

Office Action Summary	Application No. 14/615,504	Applicant(s) WATANABE ET AL.	
	Examiner SEAN MCGARRY	Art Unit 1674	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1 and 15-25* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1 and 15-25* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 1, 15, and 22-25 are rejected under 35 U.S.C. 101 because the claimed invention is not directed to patent eligible subject matter. Based upon an analysis with respect to the claim as a whole, the claims do not recite something significantly different than a judicial exception. The claimed invention reads on a fragment of a naturally occurring nucleic acid.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

Claims 1-8 and 12-14 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Popplewell et al [US20100168212], Sazani et al [US20100130591] in view of Baker et al [US20130109091] and Bennett et al [US20120190728].

The claimed invention is drawn to antisense compounds targeted to recited regions all contained within nucleotides 31-61 of exon 53 of the dystrophin gene including antisense oligomers SEQ ID NOS:4, 8, 11, 15, 18, 25, 32, 34, 36, 57, 103,-105, and 109 that cause skipping of the 53rd exon in the human dystrophin gene. The invention includes modifications to the compounds where these modifications are well known and routinely utilized in the antisense art at the time of invention.

Popplewell et al have taught antisense based alteration of splicing in the human dystrophin gene including use as pharmaceuticals. It has been taught to target exon 53 to induce skipping of the 53rd exon. The specific sequences and modifications recited in the instant claims have been clearly suggested by Popplewell et al. See for example SEQ ID NOS:10-12 and 24, and paragraph 15:

The molecule that causes skipping in exon 53 comprises at least a 25 base length from a base sequence selected from:

TABLE-US-00005 (SEQ ID NO: 10) j) CXG XXG CCX CCG GXX CXG AAG GXG XXC XXG; (SEQ ID NO: 11) k) CAA CXG XXG CCX CCG GXX CXG AAG GXG XXC; or (SEQ ID NO: 12) l) XXG CCX CCG GXX CXG AAG GXG XXC XXG XAC,

wherein the molecule's sequence can vary from the above sequence at up to two base positions, and wherein the molecule can bind to a target site to cause exon skipping in exon 53 of the dystrophin gene.

Paragraph 28:

The base "X" in the above base sequences is defined as being thymine (T) or uracil (U). The presence of either base in the sequence will still allow the molecule to bind to the pre-mRNA of the dystrophin gene as it is a complementary sequence. Therefore, the presence of either base in the molecule will cause exon skipping. The base sequence of the molecule may

contain all thymines, all uracils or a combination of the two. One factor that can determine whether X is T or U is the chemistry used to produce the molecule. For example, if the molecule is a phosphorodiamidate morpholino oligonucleotide (PMO), X will be T as this base is used when producing PMOs. Alternatively, if the molecule is a phosphorothioate-linked 2'-O-methyl oligonucleotide (2'OMePS), X will be U as this base is used when producing 2'OMePSs. Preferably, the base "X" is only thymine (T).

Paragraph 30:

The molecule can be any type of molecule as long as it has the selected base sequence and can bind to a target site of the dystrophin pre-mRNA to cause exon skipping. For example, the molecule can be an oligodeoxyribonucleotide, an oligoribonucleotide, a phosphorodiamidate morpholino oligonucleotide (PMO) or a phosphorothioate-linked 2'-O-methyl oligonucleotide (2'OMePS). Preferably, the oligonucleotide is a PMO. The advantage of a PMO is that it has excellent safety profiles and appears to have longer lasting effects in vivo compared to 2'OMePS oligonucleotides. Preferably, the molecule is isolated so that it is free from other compounds or contaminants.

Paragraph 32:

The molecule is at least 25 bases in length. Preferably, the molecule is at least 28 bases in length. Preferably, the molecule is no more than 35 bases in length and, more preferably, no more than 32 bases in length. Preferably, the molecule is between 25 and 35 bases in length, more preferably, the molecule is between 28 and 32 bases in length, even more preferably, the molecule is between 29 and 31 bases in length, and most preferably, the molecule is 30 bases in length. It has been found that a molecule which is 30 bases in length causes efficient exon skipping. If the molecule is longer than 35 bases in length, the specificity of the binding to the specific exon region is reduced. If the molecule is less than 25 bases in length, the exon skipping efficiency is reduced.

Paragraph 96:

To ensure that the analysis of PMOs for the targeted skipping of exon 53 was not biased by any particular design strategy, seventeen 25mer PMOs were designed to cover the whole of exon 53, with stepwise arrays over suggested bioactive target sites, and then subsequently six 30mer PMOs were designed to target the sequence of exon 53 that showed an association with exon skipping for the 25mers tested. PMOs were designed and tested independently by two different groups (at RHUL and UWA), and then efficacy of the best thirteen sequences confirmed by two other independent groups (at UCL and LUMC). Such a collaborative approach has been used previously as a way of validating target sequences in DMD [4]. Human myoblasts allowed the controlled in vitro comparison of PMO sequences, and confirmation of skipping of

exon 53 at the RNA level by certain PMOs in both normal cells and, perhaps more importantly, in DMD patient cells with a relevant mutation. These results were further borne out by the expression of dystrophin protein in the DMD cells treated with specific PMOs. Use of the humanised DMD mouse provided an in vivo setting to confirm correct exon exclusion prior to any planned clinical trial. The combined use of these three different systems (normal cells, patient cells and hDMD mouse) as tests of PMO bioactivity provided a reliable and coherent determination of optimal sequence(s) for the targeted skipping of exon 53.

Paragraph 97:

When considering the data presented here as a whole, the superiority of the PMO targeting the sequence +30+59 (PMO-G, or h53A30/1), is strongly indicated. In normal myoblasts, nucleofection of PMO-G (300 nM) and liposomal-carrier mediated transfection of leashed PMO-G (500 nM) produced over 80% and over 50% skipping of exon 53, respectively, implying that it acts extremely efficiently within the cell. This was confirmed in patient cells. Indeed, this PMO generates the highest levels of exon skipping in patient cells over a range of concentrations (up to 200 nM) and, most important therapeutically, exerts its activity at concentrations as low as 25 nM. The exon skipping activity of this PMO is also persistent, with over 70% exon skipping for 7 days in culture, and over 60% exon skipping for up to three weeks. This would have important safety and cost implications as a genetic therapy for DMD patients with the appropriate deletions. PMO-G was also shown to skip exon 53 correctly in vivo. These RNA results were further confirmed by the detection of dystrophin protein at a high level in protein extracts from patient cells treated with PMO-G. Previous studies by the Leiden group [18] suggest that the optimal 2'OMePS AO is targeted to the sequence +46+63 of exon 53, producing exon skipping in up to 25% of transcripts in cultured cells and 7% in the hDMD mouse. This 2'OMePS AO shows some degree of overlap with the optimal PMOs reported here which strengthens our findings. The reason that our optimal PMO is more specific could be a (combined) consequence of the different AO chemistries, length of AO used, and the absolute target site of AO.

The prior art has therefore taught that the same region targeted by the instantly claimed oligomers is superior to other regions of exon 53. The prior art has taught that sequences with SEQ ID NOS:10-12 are included in their invention. The recited SEQ ID NOS: fall squarely within SEQ ID NOS:10-12 and 24 which has been taught by Popplewell to be a “superior” target region of exon 53. While the entire document is pertinent to applicant invention, please also see Example 2 and claims 1-12.

Sazani et al have also taught antisense oligomers for inducing exon 53 skipping in the human dystrophin gene. Sazani et al have also taught oligomers targeting the same target site and the instant invention and the superior region taught by Popplewell et all See for example SEQ ID NOS: 430, 431, and 628-633 which all overlap or is/are embrace the instantly recited SEQ ID NOS. Sazani et al have also taught that oligomer size choices and modification of antisense oligonucleotides. while the entire reference is relied upon and relevant to applicants invention, applicant is directed to, for example, paragraphs 18-25, 36, 40, 50, 56, 95, 97, 98, 104, 118, 123-177, 196, 197, and claims 36-39, for example.

While the prior art has not specifically disclosed the recited sequences SEQ ID Nos, the prior art has clearly taught that such sequences are embraced within a known target region and furthermore within known antisense compounds. The prior art, however, has taught that the region that the instant compounds are targeted to is a superior target region and furthermore the prior art references taken together have taught that one in the art can alter the sizes of the antisense compounds. It would have been well within the skill of the artisan to test various sizes of oligomers in an optimization of antisense compounds targeting a known superior target region. It is noted that the superior target region is not large; "When considering the data presented here as a whole, the superiority of the PMO targeting the sequence +30+59 (PMO-G, or h53A30/1), is strongly indicated." Applicants invention is oligomers that are within this exemplified

compound where it has been clearly taught that sequences within this oligomer were considered y by the prior art. The modifications utilized in the invention and recited in the claims were well known and routinely used in the art at the time of invention as shown by the above art and evidenced by Baker et al and Bennett et al. The benefits of the modifications were well known in the art where nuclease protection, and improved hybridization, and cell penetration were known benefits, for example. Both of these references are drawn to antisense compounds utilized in alteration of splicing. See Paragraphs 10, 11, 13, 27, and 60-71 of Baker et al and paragraphs 25, 57-75, 97-104, 140-155, 176, and 180-183 of Bennett et al, for example. Bennett and Baker et al have also taught various size ranges for splice altering antisense compounds.

The invention as a whole would therefore have been *prima facie* obvious to one in the art at the time of invention.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s)

because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/forms/. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more

information about eTerminal Disclaimers, refer to

http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1 and 15-25 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-7 of U.S. Patent No. 9079934. Although the claims at issue are not identical, they are not patentably distinct from each other because the antisense oligomer of the patent overlaps significantly with and within antisense compounds targeted within or to the regions recited in the instant claim1, for example.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SEAN MCGARRY whose telephone number is (571)272-0761. The examiner can normally be reached on M-Th (7:00-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Shaojia Anna Jiang can be reached on (571) 272-0627. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Sean R McGarry
Primary Examiner
Art Unit 1674

/SEAN MCGARRY/
Primary Examiner, Art Unit 1674

Docket No.: 209658-0001-01-US-518587
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Naoki WATANABE et al.

Application No.: 14/615,504 Confirmation No.: 2704

Filed: February 6, 2015 Art Unit: 1674

For: ANTISENSE NUCLEIC ACIDS Examiner: S. McGarry

AMENDMENT / RESPONSE UNDER 37 C.F.R. § 1.111
&
PETITION FOR EXTENSION OF TIME

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the Office Action mailed March 25, 2016, Applicants submit the present Amendment / Response. The Office is respectfully requested to consider and enter the following amendments and remarks. Applicants petition herewith for a ONE-month extension of time and submit the corresponding fee, extending the period of response until July 25, 2016.

Amendments to the Abstract begin on page 2.

Amendments to the Claims begin on page 4.

Remarks begin on page 6.

AMENDMENTS TO THE ABSTRACT

Please replace the Abstract with the Substitute Abstract provided on the next page.

SUBSTITUTE ABSTRACT

~~The present invention provides a pharmaceutical composition which causes skipping of the 53rd exon in the human dystrophin gene with a high efficiency.~~

The present invention provides an oligomer which efficiently enables to cause skipping of the 53rd exon in the human dystrophin gene. Also provided is a pharmaceutical composition which causes skipping of the 53rd exon in the human dystrophin gene with a high efficiency.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of the claims in the application:

LISTING OF CLAIMS

Claim 1. (Currently Amended): An antisense oligomer which causes skipping of the 53rd exon in the human dystrophin gene, consisting of [[a]]the nucleotide sequence ~~complementary to any one of the sequences consisting of: the 31st to the 55th, the 32nd to the 53rd, the 32nd to the 56th, the 32nd to the 61st, the 33rd to the 54th, the 33rd to the 57th, the 34th to the 58th, the 35th to the 59th, the 36th to the 53rd, the 36th to the 55th, the 36th to the 57th, the 36th to the 60th, or the 37th to the 61st nucleotides from the 5' end of the 53rd exon in the human dystrophin gene~~of SEQ ID NO: 11 and SEQ ID NO: 57, wherein the antisense oligomer is an oligonucleotide in which the sugar moiety and/or the phosphate-binding region of at least one nucleotide constituting the oligonucleotide is modified, or a morpholino oligomer .

Claims 2-16. (Canceled).

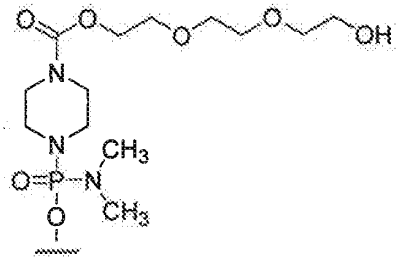
Claim 17. (Currently Amended): The antisense oligomer according to claim [[16]]1, wherein the sugar moiety of at least one nucleotide constituting the oligonucleotide is a ribose in which the 2'-OH group is replaced by any one selected from the group consisting of: OR, R, R'OR, SH, SR, NH_2, NHR, NR_2, N_3, CN, F, Cl, Br, and I, wherein R is an alkyl or an aryl and R' is an alkylene.

Claim 18. (Currently Amended): The antisense oligomer according to claim [[16]]1, wherein the phosphate-binding region of at least one nucleotide constituting the oligonucleotide is any one selected from the group consisting of: a phosphorothioate bond, a phosphorodithioate bond, an alkylphosphonate bond, a phosphoramidate bond, and a boranophosphate bond.

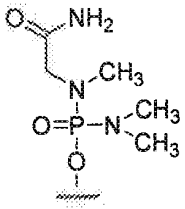
Claim 19. (Previously Presented): The antisense oligomer according to claim 1, which is a morpholino oligomer.

Claim 20. (Previously Presented): The antisense oligomer according to claim 19, which is a phosphorodiamidate morpholino oligomer.

Claim 21. (Previously Presented): The antisense oligomer according to claim 19, wherein the 5' end is any one of the groups of chemical formulae (1) to (3) below:



(1)



(2)

(3)

Claim 22. (Canceled).

Claim 23. (Currently Amended): The antisense oligomer according to claim 1, consisting of the nucleotide sequence ~~shown by any one selected from the group consisting of SEQ ID NOS: 4, 8, 11, 15, 18, 25, 32, 34, 36, 57, 103, 104, 105, and 109~~of SEQ ID NO: 57.

Claim 24. (Currently Amended): The antisense oligomer according to claim 1, consisting of the nucleotide sequence ~~shown by~~of SEQ ID NO: 11.

Claim 25. (Previously Presented): A pharmaceutical composition for the treatment of muscular dystrophy, comprising as an active ingredient the antisense oligomer according to claim 1, or a pharmaceutically acceptable salt or hydrate thereof.

REMARKS

Applicants request reconsideration in light of the above amendments and following comments submitted under 37 C.F.R. § 1.111.

1. Amendments to the Abstract

Applicants amend the Abstract to a single paragraph. No prohibited new matter is believed to be introduced.

2. Status of the Claims

The status of the claims following entry of the amendments is as follows:

Claims pending: 1, 17-21, and 23-25

Claims rejected: 1 and 15-25

Claims canceled: 2-16 and 22

Claims amended: 1, 17-18, and 22-23

Applicants amend claim 1 to recite SEQ ID NO: 11 and SEQ ID NO: 57, which respectively correspond to nucleotide sequences complementary to 36th to 60th and 32nd to 56th nucleotides from the 5' end of the 53rd exon in the human dystrophin gene. Applicants also amend claims 17-18 to update the dependency given the cancellation of claim 16. Applicants further amend claims 22 and 23 to recite SEQ ID NO: 57 and SEQ ID NO: 11, respectively. Thus, no prohibited new matter is believed to be added.

The claims have been amended without prejudice to, or disclaimer of, the canceled subject matter. Applicant reserves the right to file a continuation or divisional application on any subject matter canceled by way of amendments.

3. Information Disclosure Statements

Applicants appreciate the Office's acknowledgement of the Information Disclosure Statements (IDSs) submitted on February 6, 2015, and September 22, 2015.

4. **Status of the Drawings**

Applicants respectfully request status as to the acceptance of the drawings as filed February 6, 2015 and Replacement Sheets March 3, 2016 with the Office's next communication.

5. **Priority Documents**

Applicants respectfully request acknowledgment of the claim for foreign priority and receipt of the priority document(s) with the Office's next communication.

6. **Claim Rejection Under 35 U.S.C. § 101**

The Office rejects claims 1, 15, and 22-25 as allegedly not directed to patent eligible subject matter. Office Action, page 2. The Office alleges that (1) "the claims do no recite something significantly different than a judicial exception"; and (2) "[t]he claimed invention reads on a fragment of a naturally occurring nucleic acid." *Id.*

Upon entry of the present amendments, claims 15 and 22 stand canceled, mooting at least this aspect of the rejection. Without acquiescing as to the merits of the Office's rejection, amended independent claim 1 recites, *inter alia*, an antisense oligomer that is either (1) a modified oligonucleotide (in which the sugar moiety and/or the phosphate-binding region of at least one nucleotide constituting the oligonucleotide has been modified), or (2) a morpholino oligomer.[1] There is no evidence on the record, or adduced by the Office, that any one of the presently recited antisense oligomers would have existed as "a fragment of a naturally occurring nucleic acid." The Office's rejection is thus moot. Accordingly, Applicants respectfully request withdrawal of the rejection and allowance of claims 1 and 23-25.

7. **Claim Rejection Under 35 U.S.C. § 103(a)**

The Office rejects claims 1 and 15-25[2] as allegedly obvious over **Popplewell** et al., US 2010/0168212 ("Popplewell") and **Sazani** et al., US 2010/0130591 ("Sazani") in view of **Baker**

[1] Morpholino oligomers are synthetic molecules having standard nucleic acid bases bound to morpholine rings (instead of the deoxyribose rings in DNA). *See, e.g.*, Wikipedia page of Morpholino, *available at* http://en.wikipedia.org/wiki/Morpholino.

[2] The Office alleges "[c]laims 1-8 and 12-14" as be unpatentable over the cited references. Applicants believe that the Office must have meant "claims 1 and 15-25" as indicated in the Office Action Summary (PTOL-326).

et al., US 2013/0109091 ("Baker") and **Bennett** et al., US 2012/0190728 ("Bennett"). Office Action, pages 3-8.

Alleged Grounds for Rejection

Popplewell allegedly teaches targeting the 53rd exon of the human dystrophin gene to induce skipping of the 53rd exon. *Id.*, at 4. Popplewell's SEQ ID NOs: 10-12 and 24 allegedly suggest the presently recited sequences and modifications. *Id.*

Sazani allegedly teaches antisense oligomers for inducing exon 53 skipping in the human dystrophin gene. *Id.*, at 7. Sazani's SEQ ID NOs: 430-431 and 628-633 allegedly overlap or encompass the presently recited SEQ ID NOs: 11 or 35. *Id.*

The Office admits that none of the cited references discloses the presently recited SEQ ID NOs. *Id.*, at 8. Nevertheless, the Office alleges that "[i]t would have been well within the skill of the artisan to test various sizes of oligomers in an optimization of antisense compounds targeting a known superior target region." *Id.*

The Office by relying upon Baker and Bennett further alleges that "[t]he modifications utilized in the invention and recited in the claims were well known and routinely used in the art at the time." *Id.*, 8. The Office then concludes that "[t]he invention as whole would therefore have been prima facie obvious to one in the art at the time of invention." *Id.*

Applicants traverse the rejection to the extent it may be applied to the amended claims. Both the suggestion of the claimed invention and the expectation of success must be in the prior art, not in the disclosure of the claimed invention. *In re Dow Chem. Co.*, 837 F.2d 469 (Fed. Cir. 1988). Additionally, once the scope and content of the prior art are determined, the relevant inquiry is whether the prior art suggests all the limitations of the invention, and whether one of ordinary skill in the art would have had a reasonable expectation that the claimed invention would be successful as so combined. *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991).

Upon entry of the present amendments, independent claim 1 recites, *inter alia*, an oligomer consisting of the nucleotide sequence of SEQ ID NO: 11 or SEQ ID NO: 57. As the Office admits, none of the cited references teaches or suggests SEQ ID NO: 11 or SEQ ID NO: 57. The Office's rejection is unsupported, at least because the Office fails to articulate a rationale why a skilled artisan would have been guided or directed to modify the antisense oligomers of Sazani to arrive at the presently claimed antisense oligomers. Without such

guidance, the artisan would not have had a reasonable expectation of success in arriving at the claimed sequences. *See, e.g., In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988).[3] Teachings of Baker and Bennett are not directly applicable, because the targeted genes discussed therein differ from the human dystrophin gene.

Additionally, the presently recited oligomers (consisting of the nucleotide sequence of SEQ ID NO: 11 and 57) offer superior skipping effects over the oligomers taught in both Popplewell and Sazani. For example, Figures 2-4 of the Specification (corresponding to data in Test Examples 2-3) show that PMO No. 3 (having the nucleotide sequence of SEQ ID NO: 11; *see* Table 2) outperformed exemplary antisense oligomers taught in Popplewell (PMO Nos. 12 and 15). Additionally, Figures 18-19 of the Specification (corresponding to data in Test Example 7) show that PMO No. 3 (having the nucleotide sequence of SEQ ID NO: 11; *see* Table 2) outperformed exemplary antisense oligomer taught in Sazani (PMO No. 16).[4] Furthermore, Figures 16-17 (corresponding to data in Test Example 6) show that the oligomer having the nucleotide sequence of SEQ ID NO: 57 (H53_36-60) displays a higher level skipping activity that that having the nucleotide sequence of SEQ ID NO: 11 (H53_32-56). Thus, the recited oligomers consisting of the nucleotide sequence of SEQ ID NO: 57 also have superior skipping activity over exemplary oligomers taught in Popplewell and Sazani. Applicants submit that this superiority is unexpected, at least because none of the cited references teach or suggest such an effect.

Given at least the above arguments, claim 1 as amended and its dependent claims 17-21, and 23-25 would have been nonobvious over cited references. Claims 15-16 and 22 stand canceled, mooting at least this aspect of the rejection. Accordingly, Applicants respectfully request withdrawal of the rejection and allowance of the claims.

---

[3] "The admonition that 'obvious to try' is not the standard under § 103 has been directed mainly at two kinds of error. In some cases, what would have been 'obvious to try' would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful."

[4] Figure 19 shows that PMO No. 3 has an equivalent level of skipping activity as PMO No. 8. Figure 18 shows that PMO No. 8 has a higher level of skipping activity than the exemplary antisense oligomer taught in Sazani (PMO No. 16). Thus, a skilled artisan given Figures 18-19 would have understood the following order of the skipping activities:

PMO No. 3 ≈ PMO No. 8 >> PMO No. 16.

8. Double Patenting Rejection

The Office rejects claims 1 and 15-25 on the ground of nonstatutory double patenting as allegedly obvious over claims 1-7 of **U.S. Patent No. 9,079,934** ("the '934 patent"). Office Action, pages 8-10. The Office alleges:

> Although the claims at issue are not identical, they are not patentably distinct from each other because the antisense oligomer of the patent overlaps significantly with and within antisense compounds targeted within or to the regions recited in the instant claim 1, for example.

Id., at 10.

Without acquiescing as to the merits of the Office's rejection, Applicants amend claim 1 to recite, *inter alia*, an oligomer consisting of the nucleotide sequence of SEQ ID NO: 11 or SEQ ID NO: 57. SEQ ID NO: 11 corresponds to H53_32-56, while SEQ ID NO: 57 corresponds to H53_36-60. The '934 patent recites oligomers consisting of the nucleotide sequence of SEQ ID NO: 35, which correspond to H53_36-56. In addition to the sequence differences, the '934 patent's oligomers are shorter (21-mer) that the presently claimed oligomers (25-mer). Applicants respectfully request the Office's reconsideration given the present claim amendments.

If necessary, Applicants may consider submitting a Terminal Disclaimer Form and payment of the appropriate fee, when the obviousness-type double patenting rejection becomes the only outstanding rejection remaining.

CONCLUSION

In view of the foregoing, Applicant submits that the pending claims are in condition for allowance, and respectfully request reconsideration and timely allowance of the pending claims. Should the Examiner feel that there are any issues outstanding after consideration of this response; the Examiner is invited to contact Applicant's undersigned representative to expedite prosecution. A favorable action is awaited.

EXCEPT for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. § 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No. 50-0573. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

Dated: July 22, 2016

Respectfully submitted,

Customer Number: 055694

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/615,504	02/06/2015	Naoki WATANABE	209658-0001-01-US-518587	2704

55694 7590 10/27/2016

DRINKER BIDDLE & REATH (DC)
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WASHINGTON, DC 20005-1209

EXAMINER
MCGARRY, SEAN

ART UNIT	PAPER NUMBER
1674	

NOTIFICATION DATE	DELIVERY MODE
10/27/2016	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

DBRIPDocket@dbr.com
penelope.mongelluzzo@dbr.com

Office Action Summary	Application No. 14/615,504	Applicant(s) WATANABE ET AL.	
	Examiner SEAN MCGARRY	Art Unit 1674	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on *7/22/2016*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1,17-21,24 and 25* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1,17-21,24 and 25* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *2/6/2015 and 3/3/2015* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☒ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☒ Certified copies of the priority documents have been received in Application No. 13/819,520.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 1, 15, and 22-25 **WERE** rejected under 35 U.S.C. 101 because the claimed invention is not directed to patent eligible subject matter. Based upon an analysis with respect to the claim as a whole, the claims do not recite something significantly different than a judicial exception.

This rejection has been withdrawn in view of applicants amendments to the claims filed 7/22/2016.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which

said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

Claims 1, 17-21 and 23-25 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Popplewell et al [US20100168212], Sazani et al [US20100130591] in view of Baker et al [US20130109091] and Bennett et al [US20120190728].

The claimed invention is drawn to antisense compounds targeted to recited regions all contained within nucleotides 31-61 of exon 53 of the dystrophin gene including antisense oligomers SEQ ID NOS:4, 8, 11, 15, 18, 25, 32, 34, 36, 57, 103,-105, and 109 that cause skipping of the 53rd exon in the human dystrophin gene. The invention includes modifications to the compounds where these modifications are well known and routinely utilized in the antisense art at the time of invention.

Popplewell et al have taught antisense based alteration of splicing in the human dystrophin gene including use as pharmaceuticals. It has been taught to target exon 53 to induce skipping of the 53rd exon. The specific sequences and modifications recited in the instant claims have been clearly suggested by Popplewell et al. See for example SEQ ID NOS:10-12 and 24, and paragraph 15:

The molecule that causes skipping in exon 53 comprises at least a 25 base length from a base sequence selected from:

TABLE-US-00005 (SEQ ID NO: 10) j) CXG XXG CCX CCG GXX CXG AAG GXG XXC XXG; (SEQ ID NO: 11) k) CAA CXG XXG CCX CCG GXX CXG AAG GXG XXC; or (SEQ ID NO: 12) l) XXG CCX CCG GXX CXG AAG GXG XXC XXG XAC,

wherein the molecule's sequence can vary from the above sequence at up to two base positions, and wherein the molecule can bind to a target site to cause exon skipping in exon 53 of the dystrophin gene.

Paragraph 28:

The base "X" in the above base sequences is defined as being thymine (T) or uracil (U). The presence of either base in the sequence will still allow the molecule to bind to the pre-mRNA of the dystrophin gene as it is a complementary sequence. Therefore, the presence of either base in the molecule will cause exon skipping. The base sequence of the molecule may contain all thymines, all uracils or a combination of the two. One factor that can determine whether X is T or U is the chemistry used to produce the molecule. For example, if the molecule is a phosphorodiamidate morpholino oligonucleotide (PMO), X will be T as this base is used when producing PMOs. Alternatively, if the molecule is a phosphorothioate-linked 2'-O-methyl oligonucleotide (2'OMePS), X will be U as this base is used when producing 2'OMePSs. Preferably, the base "X" is only thymine (T).

Paragraph 30:

The molecule can be any type of molecule as long as it has the selected base sequence and can bind to a target site of the dystrophin pre-mRNA to cause exon skipping. For example, the molecule can be an oligodeoxyribonucleotide, an oligoribonucleotide, a phosphorodiamidate morpholino oligonucleotide (PMO) or a phosphorothioate-linked 2'-O-methyl oligonucleotide

(2'OMePS). Preferably, the oligonucleotide is a PMO. The advantage of a PMO is that it has excellent safety profiles and appears to have longer lasting effects in vivo compared to 2'OMePS oligonucleotides. Preferably, the molecule is isolated so that it is free from other compounds or contaminants.

Paragraph 32:

The molecule is at least 25 bases in length. Preferably, the molecule is at least 28 bases in length. Preferably, the molecule is no more than 35 bases in length and, more preferably, no more than 32 bases in length. Preferably, the molecule is between 25 and 35 bases in length, more preferably, the molecule is between 28 and 32 bases in length, even more preferably, the molecule is between 29 and 31 bases in length, and most preferably, the molecule is 30 bases in length. It has been found that a molecule which is 30 bases in length causes efficient exon skipping. If the molecule is longer than 35 bases in length, the specificity of the binding to the specific exon region is reduced. If the molecule is less than 25 bases in length, the exon skipping efficiency is reduced.

Paragraph 96:

To ensure that the analysis of PMOs for the targeted skipping of exon 53 was not biased by any particular design strategy, seventeen 25mer PMOs were designed to cover the whole of exon 53, with stepwise arrays over suggested bioactive target sites, and then subsequently six 30mer PMOs were designed to target the sequence of exon 53 that showed an association with exon skipping for the 25mers tested. PMOs were designed and tested independently by two different groups (at RHUL and UWA), and then efficacy of the best thirteen sequences confirmed by two other independent groups (at UCL and LUMC). Such a collaborative approach has been used previously as a way of validating target sequences in DMD [4]. Human myoblasts allowed the controlled in vitro comparison of PMO sequences, and confirmation of skipping of exon 53 at the RNA level by certain PMOs in both normal cells and, perhaps more importantly, in DMD patient cells with a relevant mutation. These results were further borne out by the expression of dystrophin protein in the DMD cells treated with specific PMOs. Use of the humanised DMD mouse provided an in vivo setting to confirm correct exon exclusion prior to any planned clinical trial. The combined use of these three different systems (normal cells, patient cells and hDMD mouse) as tests of PMO bioactivity provided a reliable and coherent determination of optimal sequence(s) for the targeted skipping of exon 53.

Paragraph 97:

When considering the data presented here as a whole, the superiority of the PMO targeting the sequence +30+59 (PMO-G, or h53A30/1), is strongly indicated. In normal myoblasts, nucleofection of PMO-G (300 nM) and liposomal-carrier mediated transfection of

leashed PMO-G (500 nM) produced over 80% and over 50% skipping of exon 53, respectively, implying that it acts extremely efficiently within the cell. This was confirmed in patient cells. Indeed, this PMO generates the highest levels of exon skipping in patient cells over a range of concentrations (up to 200 nM) and, most important therapeutically, exerts its activity at concentrations as low as 25 nM. The exon skipping activity of this PMO is also persistent, with over 70% exon skipping for 7 days in culture, and over 60% exon skipping for up to three weeks. This would have important safety and cost implications as a genetic therapy for DMD patients with the appropriate deletions. PMO-G was also shown to skip exon 53 correctly in vivo. These RNA results were further confirmed by the detection of dystrophin protein at a high level in protein extracts from patient cells treated with PMO-G. Previous studies by the Leiden group [18] suggest that the optimal 2'OMePS AO is targeted to the sequence +46+63 of exon 53, producing exon skipping in up to 25% of transcripts in cultured cells and 7% in the hDMD mouse. This 2'OMePS AO shows some degree of overlap with the optimal PMOs reported here which strengthens our findings. The reason that our optimal PMO is more specific could be a (combined) consequence of the different AO chemistries, length of AO used, and the absolute target site of AO.

The prior art has therefore taught that the same region targeted by the instantly claimed oligomers is superior to other regions of exon 53. The prior art has taught that sequences with SEQ ID NOS:10-12 are included in their invention. The recited SEQ ID NOS: fall squarely within SEQ ID NOS:10-12 and 24 which has been taught by Popplewell to be a “superior” target region of exon 53. While the entire document is pertinent to applicant invention, please also see Example 2 and claims 1-12.

Sazani et al have also taught antisense oligomers for inducing exon 53 skipping in the human dystrophin gene. Sazani et al have also taught oligomers targeting the same target site and the instant invention and the superior region taught by Popplewell et all See for example SEQ ID NOS: 430, 431, and 628-633 which all overlap or is/are embrace the instantly recited SEQ ID NOS. Sazani et al have also taught that oligomer size choices and modification of antisense oligonucleotides. while the entire reference is

relied upon and relevant to applicants invention, applicant is directed to, for example, paragraphs 18-25, 36, 40, 50, 56, 95, 97, 98, 104, 118, 123-177, 196, 197, and claims 36-39, for example.

While the prior art has not specifically disclosed the recited sequences SEQ ID Nos, the prior art has clearly taught that such sequences are embraced within a known target region and furthermore within known antisense compounds. The prior art, however, has taught that the region that the instant compounds are targeted to is a superior target region and furthermore the prior art references taken together have taught that one in the art can alter the sizes of the antisense compounds. It would have been well within the skill of the artisan to test various sizes of oligomers in an optimization of antisense compounds targeting a known superior target region. It is noted that the superior target region is not large; "When considering the data presented here as a whole, the superiority of the PMO targeting the sequence +30+59 (PMO-G, or h53A30/1), is strongly indicated." Applicants invention is oligomers that are within this exemplified compound where it has been clearly taught that sequences within this oligomer were considered y by the prior art. The modifications utilized in the invention and recited in the claims were well known and routinely used in the art at the time of invention as shown by the above art and evidenced by Baker et al and Bennett et al. The benefits of the modifications were well known in the art where nuclease protection, and improved hybridization, and cell penetration were known benefits, for example. Both of these references are drawn to antisense compounds utilized in alteration of splicing. See

Paragraphs 10, 11, 13, 27, and 60-71 of Baker et al and paragraphs 25, 57-75, 97-104, 140-155, 176, and 180-183 of Bennett et al, for example. Bennett and Baker et al have also taught various size ranges for splice altering antisense compounds.

The invention as a whole would therefore have been *prima facie* obvious to one in the art at the time of invention.

Response to Arguments

Applicant's arguments filed 7/22/2016 have been fully considered but they are not persuasive.

Applicant has argued that the rejection of record fails to articulate why a skilled artsen would have been guided or directed to modify the antisense oligomers of Sazani to arrive at the presently claimed oligomers. It is noted that the instant oligomers are targeted to nt32-56 and 36-60 of exon 53. These oligomers are 25mers. The examiner asserts that the rejection specifically provides the ese teachings from the prior art.

From the rejection above:

It has been taught to target exon 53 to induce skipping of the 53rd exon. The specific sequences and modifications recited in the instant claims have been clearly suggested by Popplewell et al. See for example SEQ ID NOS:10-12 and 24, and paragraph 15:

The molecule that causes skipping in exon 53 comprises at least a 25 base length from a base sequence selected from:

TABLE-US-00005 (SEQ ID NO: 10) j) CXG XXG CCX CCG GXX CXG AAG GXG XXC XXG; (SEQ ID NO: 11) k) CAA CXG XXG CCX CCG GXX CXG AAG GXG XXC; or (SEQ ID NO: 12) l) XXG CCX CCG GXX CXG AAG GXG XXC XXG XAC,

wherein the molecule's sequence can vary from the above sequence at up to two base positions, and wherein the molecule can bind to a target site to cause exon skipping in exon 53 of the dystrophin gene.

Paragraph 32:

The molecule is at least 25 bases in length. Preferably, the molecule is at least 28 bases in length. Preferably, the molecule is no more than 35 bases in length and, more preferably, no more than 32 bases in length. Preferably, the molecule is between 25 and 35 bases in length, more preferably, the molecule is between 28 and 32 bases in length, even more preferably, the molecule is between 29 and 31 bases in length, and most preferably, the molecule is 30 bases in length. It has been found that a molecule which is 30 bases in length causes efficient exon skipping. If the molecule is longer than 35 bases in length, the specificity of the binding to the specific exon region is reduced. If the molecule is less than 25 bases in length, the exon skipping efficiency is reduced.

Paragraph 97:

When considering the data presented here as a whole, the superiority of the PMO targeting the sequence +30+59 (PMO-G, or h53A30/1), is strongly indicated. In normal myoblasts, nucleofection of PMO-G (300 nM) and liposomal-carrier mediated transfection of leashed PMO-G (500 nM) produced over 80% and over 50% skipping of exon 53, respectively, implying that it acts extremely efficiently within the cell. This was confirmed in patient cells. Indeed, this PMO generates the highest levels of exon skipping in patient cells over a range of concentrations (up to 200 nM) and, most important therapeutically, exerts its activity at concentrations as low as 25 nM. The exon skipping activity of this PMO is also persistent, with over 70% exon skipping for 7 days in culture, and over 60% exon skipping for up to three weeks. This would have important safety and cost implications as a genetic therapy for DMD patients with the appropriate deletions. PMO-G was also shown to skip exon 53 correctly in vivo. These RNA results were further confirmed by the detection of dystrophin protein at a high level in protein extracts from patient cells treated with PMO-G. Previous studies by the Leiden group [18] suggest that the optimal 2'OMePS AO is targeted to the sequence +46+63 of exon 53, producing exon skipping in up to 25% of transcripts in cultured cells and 7% in the hDMD mouse. This 2'OMePS AO shows some degree of overlap with the optimal PMOs reported here which strengthens our findings. The reason that our optimal PMO is more specific could be a (combined) consequence of the different AO chemistries, length of AO used, and the absolute target site of AO.

The prior art has therefore taught that the same region targeted by the instantly claimed oligomers is superior to other regions of exon 53. The prior art has taught that sequences with SEQ ID NOS:10-12 are included in their invention. The recited SEQ ID NOS: fall squarely within SEQ ID NOS:10-12 and 24 which has been taught by Popplewell to be a “superior” target region of exon 53. While the entire document is pertinent to applicant invention, please also see Example 2 and claims 1-12.

Applicant dismissed the Baker and Bennet references since they are not directed to the same gene. This is not what the references are relied on for. For example they are relied on for what was asserted in the rejection above: modifications of antisense compounds utilized for intron splice modulation.

Applicant asserts that the instant compounds have unexpected properties. The examiner disagrees. The compounds function as designed, to alter splicing. The fact that applicant screened for more oligonucleotides in a region that has been taught to be superior utilizing size ranges and modifications known in the art is not unexpected. The prior art indeed asserts that these oligonucleotides are included in their invention as asserted in the rejection of record.

Double Patenting

Claims 1 and 15-25 **WERE** rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-7 of U.S. Patent No. 9079934.

This rejection has been withdrawn in view if the amendments filed 7/22/2016.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SEAN MCGARRY whose telephone number is (571)272-0761. The examiner can normally be reached on M-Th (7:00-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Shaojia Anna Jiang can be reached on (571) 272-0627. The fax phone

number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Sean R McGarry
Primary Examiner
Art Unit 1674

/SEAN MCGARRY/
Primary Examiner, Art Unit 1674

Docket No.: 209658-0001-01-US-518587
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Naoki WATANABE et al.

Application No.: 14/615,504 Confirmation No.: 2704

Filed: February 6, 2015 Art Unit: 1674

For: ANTISENSE NUCLEIC ACIDS Examiner: S. McGarry

AMENDMENT / RESPONSE UNDER 37 C.F.R. § 1.116
&
PETITION FOR EXTENSION OF TIME

MS AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the FINAL Office Action mailed October 27, 2016, the Office is respectfully requested to consider and enter the following amendments and remarks. Applicant petitions herewith a ONE-month extension of time, extending the period of response until February 27, 2017.

Amendments to the Claims begin on page 2.

Remarks begin on page 4.

A **Certification and Request for Consideration under the After Final Consideration Pilot Program 2.0** is concurrently submitted.

87880460.1

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of the claims in the application:

LISTING OF CLAIMS

Claim 1. (Currently Amended): An antisense oligomer which causes skipping of the 53rd exon in the human dystrophin gene, consisting of the nucleotide sequence of ~~SEQ ID NO: 11 and~~ SEQ ID NO: 57, wherein the antisense oligomer is an oligonucleotide in which the sugar moiety and/or the phosphate-binding region of at least one nucleotide constituting the oligonucleotide is modified, or a morpholino oligomer.

Claims 2-16. (Canceled).

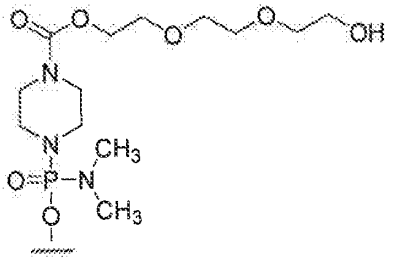
Claim 17. (Previously Presented): The antisense oligomer according to claim 1, wherein the sugar moiety of at least one nucleotide constituting the oligonucleotide is a ribose in which the 2'-OH group is replaced by any one selected from the group consisting of: OR, R, R'OR, SH, SR, NH_2, NHR, NR_2, N_3, CN, F, Cl, Br, and I, wherein R is an alkyl or an aryl and R' is an alkylene.

Claim 18. (Previously Presented): The antisense oligomer according to claim 1, wherein the phosphate-binding region of at least one nucleotide constituting the oligonucleotide is any one selected from the group consisting of: a phosphorothioate bond, a phosphorodithioate bond, an alkylphosphonate bond, a phosphoramidate bond, and a boranophosphate bond.

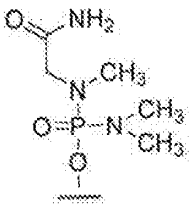
Claim 19. (Previously Presented): The antisense oligomer according to claim 1, which is a morpholino oligomer.

Claim 20. (Previously Presented): The antisense oligomer according to claim 19, which is a phosphorodiamidate morpholino oligomer.

Claim 21. (Previously Presented): The antisense oligomer according to claim 19, wherein the 5' end is any one of the groups of chemical formulae (1) to (3) below:



(1)



(2)

(3)

Claims 22-24. (Canceled).

Claim 25. (Previously Presented): A pharmaceutical composition for the treatment of muscular dystrophy, comprising as an active ingredient the antisense oligomer according to claim 1, or a pharmaceutically acceptable salt or hydrate thereof.

REMARKS

Entry of this Amendment is proper under 37 C.F.R. § 1.116, because the Amendment places the application in condition for allowance for the reasons discussed herein; does not raise any new issue requiring further search and/or consideration, because the amendments amplify issues previously discussed throughout prosecution; relates to matters of form rather than substance, because the added language was already present in the claims and thus presents no additional search burden; adds no new claims; and places the application in a better form for an appeal should an appeal be necessary. The Amendment is necessary and was not earlier presented because it is made in response to arguments raised in the final rejection. Entry of the Amendment, reexamination, and further and favorable reconsideration of the subject application given the following remarks, pursuant to and consistent with 37 C.F.R. § 1.116, are thus respectfully requested.

1. Status of the Claims and Support for the Claim Amendments

The status of the claims following entry of the amendments is as follows:

Claims pending: 1, 17-21, and 25

Claims rejected: 1, 17-21, and 23-25

Claim amended: 1

Claims canceled: 2-16 and 22-24

Applicants amend claim 1 to no longer recite SEQ ID NO: 11. Thus, no prohibited new matter is believed to be added.

The claims have been amended without prejudice to, or disclaimer of, the canceled subject matter. Applicants reserve the right to file a continuation or divisional application on any subject matter canceled by way of amendments.

2. Status the Drawings

Applicants appreciate the Office's acknowledgment that the drawings filed February 6, 2015 and March 3, 2015 are accepted by the Office.

3. **Priority Documents**

Applicants appreciates the Office's acknowledgment that certified copies of the priority document have been received in the parent application.

4. **Withdrawn Objections and Rejections**

Rejections and objections not reiterated stand withdrawn. *See* 37 C.F.R. § 1.113(b); M.P.E.P. §§ 706.07 and 707.07(e).

5. **Claim Rejection under 35 U.S.C. § 103(a)**

The Office rejects claims 1, 17-21, and 23-25 as allegedly obvious over **Popplewell** et al., U.S. Published Patent Application No. 2010/0168212 ("Popplewell") and **Sazani** et al., U.S. Published Patent Application No. 2010/0130591 ("Sazani") in view of **Baker** et al., U.S. Published Patent Application No. 2013/0109091 ("Baker") and **Bennett** et al., U.S. Published Patent Application No. 2012/0190728 ("Bennett"). Office Action, pages 2-10.

Alleged Grounds for Rejection

Popplewell allegedly teaches targeting the 53rd exon of the human dystrophin gene to induce skipping of the 53rd exon. *Id.*, at 4. Popplewell's SEQ ID NOs: 10-12 and 24 allegedly suggest the presently recited sequences and modifications. *Id.*, 4 and 8. Sazani allegedly teaches antisense oligomers for inducing exon 53 skipping in the human dystrophin gene. *Id.*, at 6-7. Sazani's SEQ ID NOs: 430-431 and 628-633 allegedly overlap or encompass the presently recited SEQ ID NOs. *Id.* The Office admits that none of the cited references discloses the presently recited SEQ ID NOs. *Id.*, at 8. Nevertheless, the Office alleges that "[i]t would have been well within the skill of the artisan to test various sizes of oligomers in an optimization of antisense compounds targeting a known superior target region." *Id.*

The Office by relying upon Baker and Bennett further alleges that "[t]he modifications utilized in the invention and recited in the claims were well known and routinely used in the art at the time." *Id.*, 7. The Office then concludes that "[t]he invention as whole would therefore have been prima facie obvious to one in the art at the time of invention." *Id.*, 8.

Given Applicants' Amendment/Response filed July 22, 2016, the Office discounts Applicants' argument. *Id.*, 10. The presently recited SEQ ID Nos "falls squarely within" what "has been taught by Popplewell to be a 'superior' target region." *Id.* The Office further alleges that "[t]he prior art indeed asserts that these oligonucleotides are included in their invention as asserted in the rejection of record." *Id.*

Arguments

Applicants traverse the rejection to the extent it may be applied to the amended claims. Both the suggestion of the claimed invention and the expectation of success must be in the prior art, not in the disclosure of the claimed invention. *In re Dow Chem. Co.*, 837 F.2d 469 (Fed. Cir. 1988). Additionally, once the scope and content of the prior art are determined, the relevant inquiry is whether the prior art suggests all the limitations of the invention, and whether one of ordinary skill in the art would have had a reasonable expectation that the claimed invention would be successful as so combined. *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991).

Upon entry of the present amendments, independent claim 1 recites, *inter alia*, an oligomer consisting of the nucleotide sequence of SEQ ID NO: 57. As the Office admits, none of the cited references teaches or suggests SEQ ID NO: 57. The Office's rejection is unsupported, at least because the Office fails to articulate a rationale why a skilled artisan would have been guided or directed to modify the antisense oligomers of the cited references to arrive at the presently claimed antisense oligomers. Without such guidance, the artisan would not have had a reasonable expectation of success in arriving at the claimed sequences. *See, e.g., In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988).[1] Teachings of Baker and Bennett are not directly applicable, because the targeted genes discussed therein differ from the human dystrophin gene.

In fact, a skilled artisan given Popplewell would have been directed to use or modify the oligomers that are different from the presently recited ones. Popplewell teaches that the oligomer corresponding to positions 30-59 of exon 53 provides the highest activity. *See, e.g.,*

[1] "The admonition that 'obvious to try' is not the standard under § 103 has been directed mainly at two kinds of error. In some cases, what would have been 'obvious to try' would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful."

Popplewell, ¶ [0074][2] and FIG. 8. In contrast, the presently recited SEQ ID NO: 57 corresponds to positions 36-60 of exon 50. Thus, Popplewell's top performer is different from the presently recited ones. There is no evidence on the record, or adduced by the Office, that a skilled artisan given Popplewell's teachings would have arrived at the presently recited oligomers, let alone doing so with any expectation of success.

Additionally, the presently recited oligomer (consisting of the nucleotide sequence of SEQ ID NO: 57) offer superior skipping effects over the oligomers taught in both Popplewell and Sazani. For example, Figures 2-4 of the Specification (corresponding to data in Test Examples 2-3) show that PMO No. 3 (having the nucleotide sequence of SEQ ID NO: 11; *see* Table 2) outperformed exemplary antisense oligomers taught in Popplewell (PMO Nos. 12 and 15). As shown in TABLE 2, PMO Nos. 12 and 15 corresponds to the top performer taught in Popplewell (targeting sequence 30-59 of exon 53). Additionally, Figures 18-19 of the Specification (corresponding to data in Test Example 7) show that PMO No. 3 (having the nucleotide sequence of SEQ ID NO: 11; *see* Table 2) outperformed exemplary antisense oligomer taught in Sazani (PMO No. 16).[3] Furthermore, Figures 16-17 (corresponding to data in Test Example 6) show that the oligomer having the nucleotide sequence of SEQ ID NO: 57 (H53_36-60) displays a higher level skipping activity that that having the nucleotide sequence of SEQ ID NO: 11 (H53_32-56). Thus, the recited oligomers consisting of the nucleotide sequence of SEQ ID NO: 57 also have superior skipping activity over exemplary oligomers taught in Popplewell and Sazani, particularly the top performer taught in Popplewell. Applicants submit that this superiority is unexpected, at least because none of the cited references teach or suggest such an effect.

Given at least the above arguments, claim 1 as amended and its dependent claims 17-21, and 25 would have been nonobvious over cited references. Claims 23-24 stand canceled,

---

2 "…produced the most robust skipping of exon 53, and should be considered the sequence of choice for any upcoming PMO clinical trial."

3 Figure 19 shows that PMO No. 3 has an equivalent level of skipping activity as PMO No. 8. Figure 18 shows that PMO No. 8 has a higher level of skipping activity than the exemplary antisense oligomer taught in Sazani (PMO No. 16). Thus, a skilled artisan given Figures 18-19 would have understood the following order of the skipping activities:

PMO No. 3 ≈ PMO No. 8 >> PMO No. 16.

mooting at least this aspect of the rejection. Accordingly, Applicants respectfully request withdrawal of the rejection and allowance of the claims.

CONCLUSION

In view of the foregoing, Applicants submit that the pending claims are in condition for allowance, and respectfully request reconsideration and timely allowance of the pending claims. Should the Office have any questions or comments regarding Applicants' amendments or response, please contact Applicants' undersigned representative at (202) 230-5119. Furthermore, please direct all correspondence to the below-listed address.

EXCEPT for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. § 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No. 50-0573. If an Appeal fee is required to maintain pendency of the present application, the Office is authorized to charge the Appeal fee to the deposit account above and use this paper as a constructive Notice of Appeal.

Dated: February 27, 2017

Respectfully submitted,

Customer Number: 055694

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NOTICE OF ALLOWANCE AND FEE(S) DUE

55694 7590 03/10/2017
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WASHINGTON, DC 20005-1209

EXAMINER	
MCGARRY, SEAN	
ART UNIT	PAPER NUMBER
1674	

DATE MAILED: 03/10/2017

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/615,504	02/06/2015	Naoki WATANABE	209658-0001-01-US-518587	2704

TITLE OF INVENTION: ANTISENSE NUCLEIC ACIDS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	06/12/2017

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

55694 7590 03/10/2017
DRINKER BIDDLE & REATH (DC)
1500 K STREET, N.W.
SUITE 1100
WASHINGTON, DC 20005-1209

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/615,504	02/06/2015	Naoki WATANABE	209658-0001-01-US-518587	2704

TITLE OF INVENTION: ANTISENSE NUCLEIC ACIDS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	06/12/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
MCGARRY, SEAN	1674	536-024500

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
- ☐ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☐ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
  NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
  NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ☐ Applicant changing to regular undiscounted fee status.
  NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/615,504	02/06/2015	Naoki WATANABE	209658-0001-01-US-518587	2704

55694 7590 03/10/2017

DRINKER BIDDLE & REATH (DC)
1500 K STREET, N.W.
SUITE 1100
WASHINGTON, DC 20005-1209

EXAMINER	
MCGARRY, SEAN	

ART UNIT	PAPER NUMBER
1674	

DATE MAILED: 03/10/2017

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 14/615,504	Applicant(s) WATANABE ET AL.	
	Examiner SEAN MCGARRY	Art Unit 1674	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *AF filed 2/27/2017*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *7,17-21 and 25*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☒ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☒ Certified copies of the priority documents have been received in Application No. *13/819,520* .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☐ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☒ Other *PTO-2323*.

/SEAN MCGARRY/
Primary Examiner, Art Unit 1674

AFCP 2.0 Decision	Application No.	Applicant(s)
	14/615,504	WATANABE ET AL.
	Examiner	Art Unit
	SEAN MCGARRY	1674

This is in response to the After Final Consideration Pilot request filed 2/27/2017.

1. **Improper Request** – The AFCP 2.0 request is improper for the following reason(s) and the after final amendment submitted with the request will be treated under pre-pilot procedure.

☐ An AFCP 2.0 request form PTO/SB/434 (or equivalent document) was not submitted.

☐ A non-broadening amendment to at least one independent claim was not submitted.

☐ A proper AFCP 2.0 request was submitted in response to the most recent final rejection.

☐ Other:

2. **Proper Request**

A. After final amendment submitted with the request will not be treated under AFCP 2.0.
The after final amendment cannot be reviewed and a search conducted within the guidelines of the pilot program.

☐ The after final amendment will be treated under pre-pilot procedure.

B. Updated search and/or completed additional consideration.
The examiner performed an updated search and/or completed additional consideration of the after final amendment within the time authorized for the pilot program. The result(s) of the updated search and/or completed additional consideration are:

☒ 1. All of the rejections in the most recent final Office action are overcome and a Notice of Allowance is issued herewith.

☐ 2. The after final amendment would not overcome all of the rejections in the most recent final Office action. See attached interview summary for further details.

☐ 3. The after final amendment was reviewed, and it raises a new issue(s). See attached interview summary for further details.

☐ 4. The after final amendment raises new issues, but would overcome all of the rejections in the most recent final Office action. A decision on determining allowability could not be made within the guidelines of the pilot. See attached interview summary for further details, including any newly discovered prior art.

☐ 5. Other:

Examiner Note: Please attach an interview summary when necessary as described above.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/615,504	07/18/2017	9708361	209658-0001-01-US-518587	2704

55694 7590 06/28/2017

DRINKER BIDDLE & REATH (DC)
1500 K STREET, N.W.
SUITE 1100
WASHINGTON, DC 20005-1209

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Naoki WATANABE, Tsukuba-shi, JAPAN;
NIPPON SHINYAKU CO., LTD., Kyoto-shi, JAPAN;
Youhei SATOU, Tsukuba-shi, JAPAN;
NATIONAL CENTER OF NEUROLOGY AND PSYCHIATRY, Tokyo, JAPAN;
Shin'ichi TAKEDA, Tokyo, JAPAN;
Tetsuya NAGATA, Tokyo, JAPAN;

EXHIBIT AJ

8145738

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

August 12, 2021

THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF THE FILE WRAPPER AND CONTENTS OF:

APPLICATION NUMBER: *16/359,213*
FILING DATE: *March 20, 2019*
PATENT NUMBER: *10385092*
ISSUE DATE: *August 20, 2019*

Certified by

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PTO/SB/08b (07-09)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	19	of	21

Complete if Known	
Application Number	16/359,213
Filing Date	March 20, 2019
First Named Inventor	Naoki WATANABE
Art Unit	1635
Examiner Name	S. McGarry
Attorney Docket Number	209658-0001-03-US-585479

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CM	Marcusson et al., MOLECULAR BIOTECHNOLOGY, Volume 12, 1999, 1-11	
	CN	Patentee's argument filed with JPO in JP Appl'n 2013-260728 on Apr.13, 2015	
	CO	Decision of Rejection by JPO in JP Appl'n 2011-098952 on Aug. 21, 2013	
	CP	Patentee's argument filed with the JPO in Opposition of JP6126983 on Mar. 23, 2016 (w/ English translation)	
	CQ	David R Corey et al Genome Biology 2001 2(5) 1015.1-1015.3	
	CR	AU 2004903474 filed Jun. 28, 2004, priority document for PCT/AU05/000943	
	CS	Experimental report submitted in EPO Opposition in EP 2206781, Aug. 25, 2016	
	CT	Experimental report (D 8-1) submitted in EPO Opposition in EP 2206781, Sept. 29, 2017	
	CU	Map of target region, submitted in EPO Opposition in EP 2206781, Feb. 22, 2017	
	CV	Experimental report, submitted in EPO Opposition in EP 2206781, Feb. 22, 2017	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08b (07-09)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	20	of	21

Complete if Known	
Application Number	16/359,213
Filing Date	March 20, 2019
First Named Inventor	Naoki WATANABE
Art Unit	1635
Examiner Name	S. McGarry
Attorney Docket Number	209658-0001-03-US-585479

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CW	Declaration by Fred Schnell, dated Sept. 28, 2017, submitted in EPO Opposition in EP 2206781, Sept. 29, 201756	
	CX	Summerton et al Antisense&Nucleic acid drug development 7:187-195(1997)	
	CY	Experimental report (D13), submitted in EPO Opposition in EP 2206781, Sept. 29, 2017	
	CZ	Declaration by Fred Schnell submitted in EP2206781 Opposition on Apr. 25, 2018	
	DA	Amendment in response to Non-Final Office Action in US 15/705,172, filed Jan 5, 2018	
	DB	UNIVERSITY OF WESTERN AUSTRALIA MOTION 1 filed in U.S. Patent Interference No. 106,007 (RES), on Nov. 18, 2014	
	DC	Prior et al., Human Genetics 92: 302-304 (1992)	
	DD	Abstracts: 32nd European Muscle Conference, 'A link between fundamental research and therapeutic trials,' The Annual Meeting of the European Society for Muscle Research, Journal of Muscle Research and Cell	
	DE	Wells et al., FEBS LETT. 2003 vol.552 145-149	
	DF	Cagliani et al., Human Genetics June 2004 Vol.115 13-18	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

PATENT
ATTORNEY DOCKET NO. 209658-0001-03-US-585479

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Naoki WATANABE et al.	Allowed: June 4, 2019
Application No.: 16/359,213	Confirmation No.: 6897
Filed: March 20, 2019	Art Unit: 1635
For: ANTISENSE NUCLEIC ACIDS	Examiner: S. McGarry

INFORMATION DISCLOSURE STATEMENT (IDS)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

☒ **Under 37 C.F.R. § 1.97(b):** Pursuant to 37 C.F.R. §§ 1.56 and 1.97(b), Applicant brings to the attention of the Examiner the documents listed on the attached PTO/SB/08 Form. To the undersigned's knowledge, this IDS is being filed before the mailing date of a first Office Action on the merits, before the mailing date of a first Office Action on the merits after filing an RCE under § 1.114, or within three months of the application filing date.

☐ **Under 37 C.F.R. § 1.97(c):** Pursuant to 37 C.F.R. §§ 1.56 and 1.97(c), Applicant brings to the attention of the Examiner the documents listed on the attached PTO/SB/08 Form. This IDS is being filed after the events recited in § 1.97(b) but, to the undersigned's knowledge, before the mailing date of a Final Office Action, a Notice of Allowance, or another action that closes prosecution in the application.

☐ The fee of $240.00 set forth in § 1.17(p) is included herein;

☐ Applicant submits that each item of information contained in this IDS was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this IDS. 37 C.F.R. § 1.97(e)(1).

☐ Applicant submits that no item of information contained in this IDS was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in this IDS was known to any individual designated in § 1.56(c) more than three months prior to the filing of this IDS. 37 C.F.R. § 1.97(e)(2).

☐ **Under 37 C.F.R. § 1.97(d):** Pursuant to 37 C.F.R. §§ 1.56 and 1.97(d), Applicant brings to the attention of the Examiner the documents listed on the attached PTO/SB/08 Form. This IDS is being filed after the events recited in § 1.97(c) but before payment of the issue fee.

☐ The fee of $240.00 set forth in § 1.17(p) is included herein; and

☐ Applicant submits that each item of information contained in this IDS was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this IDS.

☐ Applicant submits that no item of information contained in this IDS was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in this IDS was known to any individual designated in § 1.56(c) more than three months prior to the filing of this IDS. 37 C.F.R. § 1.97(e)(2).

☐ **Under 37 C.F.R. § 1.97(i):** Pursuant to 37 C.F.R. §§ 1.56 and 1.97(i), Applicant brings to the attention of the Examiner the documents listed on the attached PTO/SB/08 Form. This IDS is being filed after the events recited in § 1.97(d). Applicant requests that the IDS be placed in the file.

☐ A search report or other listing of documents from a counterpart, related, or other application dated and having documents cited thereon is attached for the Examiner's consideration. Any of these documents not previously cited, and any additional documents are listed on the PTO/SB/08 Form.

☒ Applicant respectfully requests that the Examiner consider the listed documents and evidence that consideration by making appropriate notations on the attached form. As for any document listed on the accompanying PTO/SB/08 Form that is in a language other than English, relevance can be understood from an enclosed English abstract or corresponding English-language document or at least partial translation or from mention in the specification or in a search report for a corresponding application.

This submission does not represent that a search has been made or that no better art exists and does not constitute an admission that any of the listed documents are material or constitute "prior art." If it should be determined that any of the listed documents do not constitute "prior art" under United States law, Applicant reserves the right to present to the Office the relevant facts and law regarding the appropriate status of such documents.

Applicant further reserves the right to take appropriate action to establish the patentability of the disclosed invention over the listed documents, should any of the documents be applied against the claims of the present application.

Except for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this Application, including fees due under 37 C.F.R. § 1.16 and 1.17 which may be required and including any required extension of time fees, or credit any overpayment to Deposit Account No. 50-0573. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

Respectfully submitted,

DRINKER BIDDLE & REATH LLP

Zhengyu Feng, Ph.D.
Registration No.: 66,816
DATED: June 7, 2019

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EXHIBIT AK

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

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Complete if Known	
Application Number	Not Yet Assigned
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First Named Inventor	Naoki WATANABE
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	209658-0001-11-US-000003

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	A	US-6,653,467	11-25-2003	Matsuo et al.	
	B	US-6,727,355	04-27-2004	Matsuo et al.	
	C	US-8,084,601	12-27-2011	Popplewell et al.	
	D	US-8,455,636	06-04-2013	Wilton et al.	
	E	US-8,871,918	10-28-2014	Sazani et al.	
	F	US-9,024,007	05-05-2015	Wilton et al.	
	G	US-9,994,851-B2	06-12-2018	Wilton et al.	
	H	US-10,112,977	10-30-2018	Baileykobayashi et al.	
	I	US-10,266,827	04-23-2019	Wilton et al.	
	J	US-10,227,590	03-12-2019	Wilton et al.	
	K	US-2006/0147952-A1	07-06-2006	van Ommen et al.	
	L	US-2010/0168212-A1	07-01-2010	POPPLEWELL et al.	
	M	US-2012/0190728-A1	07-26-2012	Bennett et al.	
	N	US-2013/0072541-A1	03-21-2013	Garcia	
	O	US-2013/0109091-A1	05-02-2013	Baker et al.	
	P	US-2017/0218019-A1	08-03-2017	Baileykobayashi et al.	
	Q	US-2019/0127738-A1	05-02-2019	SAZANI et al.	

FOREIGN PATENT DOCUMENTS

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	A**	CA-2507125-A1	06-10-2004	Matsuo, Masafumi		
	B**	EP-1054058-A1	11-22-2000	Jcr Pharmaceuticals Co., Ltd		
	C**	EP-1160318-A2	12-05-2001	Jcr Pharmaceuticals Co., Ltd		
	D**	EP-1191097-A1	03-27-2002	Leids Uni Medisch Ct		
	E**	EP-1191098-A2	03-27-2002	Jcr Pharmaceuticals Co., Ltd		
	F**	EP-1568769-A1	08-31-2005	Matsuo, Masafumi		

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	G**	EP-2206781-A2	07-14-2010	Univ Western Australia		
	H**	EP-2594640-B1	12-30-2015	Academisch Ziekenhuis Leiden		
	I**	EP-2602322-A1	06-12-2013	Academisch Ziekenhuis Leiden		
	J**	EP-2602322-B1	03-02-2016	Academisch Ziekenhuis Leiden		
	K**	EP-3404100-A1	11-21-2018	Sarepta Therapeutics, Inc		
	L**	JP-2000-325085-A	11-28-2000	Matsuo, Masafumi		

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	M**	JP-2002-10790-A	01-15-2002	Matsuo Masafumi		√
	N**	JP-2002-325582-A	11-12-2002	Matsuo, Masafumi		
	O**	JP-6406782-B2	10-17-2018			√
	P**	WO-02/24906-A1	03-28-2002	Academisch Ziekenhuis Leiden		
	Q**	WO-03/095647-A2	11-20-2003	Univ Roma		
	R**	WO-2004/048570-A1	06-10-2004	Univ Kobe		

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	S**	WO-2004/083432-A1	09-30-2004	Academisch Ziekenhuis Leiden		
	T**	WO-2004/083446-A2	09-30-2004	Academisch Ziekenhuis Leiden		
	U**	WO-2006/000057-A1	01-05-2006	Univ Western Australia		
	V**	WO-2006/017522-A2	02-16-2006	Univ Utah Res Found		
	W**	WO-2006/112705-A2	10-26-2006	Academisch Ziekenhuis Leiden		
	X**	WO-2007/135105-A1	11-29-2007	Academisch Ziekenhuis Leiden		

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	Y**	WO-2008/036127-A2	03-27-2008	Avi Biopharma, Inc		
	Z**	WO-2009/054725-A2	04-30-2009	Academisch Ziekenhuis Leiden		
	AA**	WO-2009/139630-A2	11-19-2009	Prosensa Technologies BV		
	AB**	WO-2010/048586-A1	04-29-2010	Avi Biopharma, Inc		
	AC**	WO-2010/050801-A1	05-06-2010	Prosensa Technologies BV		
	AD**	WO-2010/050802-A2	05-06-2010	Academisch Ziekenhuis Leiden		

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	AE**	WO-2010/123369-A1	10-28-2010	Prosensa Technologies BV		
	AF**	WO-2011/057350-A1	05-19-2011	Univ Western Australia		
	AG**	WO-2012/109296-A1	08-16-2012	Charlotte Mecklenburg Hospital		
	AH**	WO-2012/150960-A1	11-08-2012	Avi Biopharma, Inc		
	AI**	WO-2013/112053-A1	08-01-2013	Prosensa Technologies BV		
	AJ**	WO-2014/007620-A2	01-09-2014	Prosensa Technologies BV		

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	AK**	WO-2014/100714-A1	06-26-2014	Sarepta Therapeutics, Inc		
	AL**	WO-2014/144978-A2	09-18-2014	Sarepta Therapeutics, Inc		
	AM**	WO-2014/153220-A2	09-25-2014	Sarepta Therapeutics, Inc		
	AN**	WO-2014/153240-A2	09-25-2014	Sarepta Therapeutics, Inc		
	AO**	WO-2015/199039-A1	12-30-2015	Toagosei Co Ltd		
	AP**	WO-2016/025339-A2	02-18-2016	Res Inst Nationwide Childrens Hospital		

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	AQ**	WO-2017/059131-A1	04-06-2017	Sarepta Therapeutics, Inc		
	AR**	WO-2017/062835-A2	04-13-2017	Sarepta Therapeutics, Inc		
	AS**	WO-2017/205496-A1	11-30-2017	Sarepta Therapeutics, Inc		
	AT**	WO-2017/205513-A1	11-30-2017	Sarepta Therapeutics, Inc		
	AU**	WO-2017/205879-A3	01-18-2018	Sarepta Therapeutics, Inc		
	AV**	WO-2017/205879-A2	11-30-2017	Sarepta Therapeutics, Inc		

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	AW**	WO-2017/205880-A1	11-30-2017	Sarepta Therapeutics, Inc		
	AX**	WO-2017/213854-A1	12-14-2017	Sarepta Therapeutics, Inc		
	AY**	WO-2018/005805-A1	01-04-2018	Sarepta Therapeutics, Inc		
	AZ**	WO-2018/091544-A1	05-24-2018	Biomarin Pharmaceutical, Inc		
	BA**	WO-2018/118599-A1	06-28-2018	Sarepta Therapeutics, Inc		
	BB**	WO-2018/118627-A1	06-28-2018	Sarepta Therapeutics, Inc		

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	10	of	26

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	Concurrently Herewith
First Named Inventor	Naoki WATANABE
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	209658-0001-11-US-000003

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	BC**	WO-2018/118662-A1	06-28-2018	Sarepta Therapeutics, Inc		
	BD**	WO-2019/046755-A1	03-07-2019	Sarepta Therapeutics, Inc		
	BE**	WO-2019/067975-A1	04-04-2019	Sarepta Therapeutics, Inc		
	BF**	WO-2019/067979-A1	04-04-2019	Sarepta Therapeutics, Inc		
	BG**	WO-2019/067981-A1	04-04-2019	Sarepta Therapeutics, Inc		
	BH**	WO-99/053101-A1	10-21-1999	Isis Pharmaceuticals, Inc		

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	A**	LINDA J. POPPLEWELL et al., "Design of Phosphorodiamidate Morpholino Oligomers (PMOs) for the Induction of Exon Skipping of the Human *DMD* Gene," Mol. Ther., Vol. 17, no. 3, March 2009, pp. 554-561.	
	B**	LINDA J. POPPLEWELL et al., "Comparative analysis of antisense oligonucleotide sequences targeting exon 53 of the human *DMD* gene: Implications for future clinical trials," Neuromuscular Disorders, Vol. 20, No. 2, 2010.02, pp. 102-110.	
	C**	ANNEMIEKE AARTSMA-RUS et al., "Targeted exon skipping as a potential gene correction therapy for Duchenne muscular dystrophy," Neuromuscular Disorders, Vol. 12, 2002, pp. S71-S77.	
	D**	STEVE D. WILTON et al., "Antisense Oligonucleotide-induced Exon Skipping Across the Human Dystrophin Gene Transcript," Mol Ther., Vol. 15, No. 7, July 2007, pp. 1288-1296.	
	E**	ANTHONY P. MONACO et al., "An Explanation for the Phenotypic Differences between Patients Bearing Partial Deletions of the DMD Locus," Genomics, 1988; 2, pp. 90-95.	
	F**	MASAFUMI MATSUO, "Duchenne / Becker muscular dystrophy: from molecular diagnosis to gene therapy," Brain & Development, 1996; 18, pp. 167-172.	
	G**	International Search Report dated October 11, 2011 in PCT/JP2011/070318 filed August 31, 2011.	
	H**	Mitrpant, et al., "By-passing the nonsense mutation in the 4^{CV} mouse model of muscular dystrophy by induced exon skipping", The Journal of Gene Medicine, January 2009, vol. 11, No. 1, pp, 46-56	
	I**	Ito, et al., "Purine-Rich Exon Sequences Are Not Necessarily Splicing Enhancer Sequence in the Dystrophin Gene," Kobe J. Med. Sci. 47, October 2001, pp. 193-202	
	J**	Muntoni, et al., "Dystrophin and mutations: one gene, several proteins, multiple phenotypes," THE LANCET Neurology, December 2003, Vol. 2, pp. 731-740	

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	K**	Muntoni, et al., "128th ENMC International Workshop on 'Preclinical optimization and Phase I/II Clinical Trials Using Antisense Oligonucleotides in Duchenne Muscular Dystrophy' 22-24 October 2004, Naarden, The Netherlands," Neuromuscular Disorders, 2005, Vol. 15, pp. 450-457	
	L**	Pramono et al BBRC 226 (1996) 445-449	
	M**	Tanaka et al Mol Cell Biol 1994, 1347-54	
	N**	Arechavala-Gomeza et al Hum Gen Thr 2007 798-810	
	O**	Aartsma-Rus et al Mol Ther 2009 17(3): 548-553	
	P**	Wu et al PLoS One 2011 e19906	
	Q**	Declaration by Matthew J.A. Wood executed Nov. 18, 2014 in U.S. Patent Interference Nos. 106,007, 106,008, 106,113	
	R**	Sherratt et al Am J Hum Genet 1193 1007-15	
	S**	Roberts et al Lancet 1990 1523-26	
	T**	Roberts et al Hum Mut 1994 1-11	

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	U**	Roberts et al Genomics 1993 536-538	
	V**	Dunckley et al Hum Mol Genet 1995, 1083-90	
	W**	Shiga et al J Clin Invest 1997 2204-10	
	X**	Wilton et al Neuromuscul Disord 1999, 330-8	
	Y**	Coulter et al Mol Cell Biol 1997 2143-50	
	Z**	Tian and Kole Mol Cell Biol 1995 6291-98	
	AA**	Liu et al Gen&Dev 1998 1998-2012	
	AB**	Applicant's letter to EPO in EP Application No. 12198517.0, dated Dec. 9, 2013	
	AC**	Applicant's letter to EPO in EP Application No. 10177969.2, dated Mar. 7, 2016	
	AD**	Ito et al., Journal of Japanese Society for Inherited Metabolic Diseases, Vol. 15, No. 2, Nov. 1999, p162 (w/ English translation)	

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	AE**	Annex B of Applicant's letter to EPO in EP Application No. 10177969.2, dated Mar. 7, 2016	
	AF**	Patentee's letter in EPO Opposition of EP 1619249, T1383/13-3.3.08, dated Jun. 10, 2014	
	AG**	Patentee's letter in EPO Opposition of EP 1619249, T1383/13-3.3.08, dated Jan. 8, 2014	
	AH**	Deposition of Judith van Deutekom dated Mar. 11, 2015, in U.S. Patent Interference Nos. 106,007, 106,008	
	AI**	FDA Briefing Document, Nov. 24, 2015	
	AJ**	Artsma-Rus et al Hum Mol Genet 2003, 907-14	
	AK**	Van Deutekom N Eng J Med 2007 2677-86	
	AL**	Van Deutekom et al Hum Mol Genet 2001, 1547-54	
	AM**	Takeshima et al, JSHG 1999, the 44th Annual Meeting of the Japan Society of Human Genetics, Abstract, p83 (WC9) (w/ English translation)	
	AN**	Takeshima et al, Journal of Japanese Society for Inherited Metabolic Diseases, Vol. 15, No. 2, No. 1999, p163 (101) (w/ English translation)	

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	AO**	English Translation of JP2000-125448 filed Apr. 26, 2000, Priority document of EP1160318	
	AP**	EPO register for EP1160318, obtained Nov. 14, 2016	
	AQ**	Mann et al J Gen Med 2002 644-54	
	AR**	Declaration by Judith van Deutekom executed Feb. 16, 2015 in U.S. Patent Interference No. 106,007	
	AS**	BioMarin Press Release, May 31, 2016	
	AT**	Wilton & Fletcher Acta Myol 2005 222-9	
	AU**	Aartsma-Rus & Ommen 2007 1609-24	
	AV**	Heemskerk et al J Gen Med 2009 257-66	
	AW**	Chan et al Clin Exp Phar Phys 2006 533-540	
	AX**	Jarver et al Nuc Acid Ther 2014 37-47	

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Sheet	16	of	26	Attorney Docket Number	209658-0001-11-US-000003

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Filing Date	Concurrently Herewith
First Named Inventor	Naoki WATANABE
Art Unit	N/A
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	AY**	Aartsma-Rus et al Gen Thr 2004 1391-8	
	AZ**	Decision in U.S. Patent Interference No. 106,007, entered May 12, 2016	
	BA**	Withdrawal and Reissue of Decision on Motions in U.S. Patent Interference No. 106,007, entered May 12, 2016	
	BB**	Errata in U.S. Patent Interference No. 106,007, entered May 23, 2016	
	BC**	English Translation of JP2000-256547, filed Aug. 25, 2000, Priority document of EP1191098	
	BD**	Interlocutory decision in Opposition proceedings for EP1619249B, issued Apr. 15, 2013	
	BE**	EPO Office Action issued in EP Application No. 01979073.2 (EP 1320597) Jan. 7, 2015	
	BF**	Takeshima et al J Clin Invest 1995, 515-20	
	BG**	Experimental Report submitted in EP Opposition Proceeding of EP 2602322, Nov. 28, 2016	
	BH**	Takeshima et al Brain Dev 2001, 788-90	

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	BI**	Karras et al, Mol Pharm 2000, 380-7	
	BJ**	Wang et al PNAS 2000, 13714-9	
	BK**	Watakabe et al Genes&Dev 1993, 407-18	
	BL**	Lehninger, Principles of Biochemistry, 2000 3rd Edition, pages 330-331	
	BM**	Artsma-Rus et al Oligonucleotides 2010, 1-9	
	BN**	Statement of Grounds of Appeal submitted in EP 1619249 B1, Aug. 23, 2013	
	BO**	Artsma-Rus et al Oligonucleotides 2005, 284-97	
	BP**	Letter submitted to EPO in EP 12198485.0, dated Oct. 23 2014	
	BQ**	Experimental Report (comparative analysis of AONs for inducing the skipping exon 45) submitted in EP Opposition Proceeding of EP 2602322, May 22, 2017	
	BR**	Decision of Opposition Division in EP 1619249 (EP Application No. 05076770.6), issued Apr. 15, 2013	

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	BS**	Reply to the Grounds of Appeal in EP 1619249 (EP Application No. 05076770.6), dated Jan. 8, 2014	
	BT**	Experimental Report (In Silico -Wilton sequence) submitted in EP Opposition Proceeding of EP 2602322, May 22, 2017	
	BU**	Comparative study on exon 44 submitted in Opposition Proceeding of EP 2602322, May 22, 2017	
	BV**	Comparative study on exon 45 submitted in Opposition Proceeding of EP 2602322, May 22, 2017	
	BW**	Comparative study on exon 52 submitted in Opposition Proceeding of EP 2602322, May 22, 2017	
	BX**	Comparative study on exon 53 submitted in Opposition Proceeding of EP 2602322, May 22, 2017	
	BY**	CV of Judith van Deutekom submitted in Opposition Proceeding of EP 2602322, May 22, 2017	
	BZ**	Letter to EPO in EP 2602322 (EP Application No. 12198517.0) dated Oct. 21, 2014	
	CA**	Declaration by Judith van Deutekom submitted in Opposition Proceeding of EP 2602322, May 22, 2017	
	CB**	Declaration by Judith van Deutekom submitted in Opposition Proceeding of EP 2602322, Apr. 20, 2018	

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	CC**	EPO Office Action in EP Application No. 12198517.0, dated Feb. 25, 2015	
	CD**	Expert declaration by Judith van Deutekom submitted in Opposition Proceeding of EP 2602322, Apr. 20, 2018	
	CE**	Map of AONs and Exon 53, submitted in Opposition Proceeding of EP 2602322, Apr. 20, 2018	
	CF**	Evidence regarding inventorship assignment, screenshot search in the online Business Register of The Netherlands Chamber of Commerce for Leids Universitair Medisch Centrum, submitted in EP Opposition	
	CG**	Evidence regarding inventorship assignment, screenshot search in the online Business Register of The Netherlands Chamber of Commerce for Academisch Ziekenhuis Leiden, submitted in EP Opposition	
	CH**	Evidence regarding inventorship assignment, digitally certified extract from the Business Register of The Netherlands Chamber of Commerce, submitted in EP Opposition Proceeding of EP 2602322, May 23, 2018	
	CI**	Declaration by Huibert Jacob Houtkooper, submitted in Opposition Proceeding of EP 2602322, Mar. 14, 2019	
	CJ**	Declaration of Lambert Oosting, submitted in Opposition Proceeding of EP 2602322, Mar. 14, 2019	
	CK**	JPO Decision to maintain JP Patent No. 6126983 (w/ partial English translation), submitted in Opposition Proceeding of EP 2602322, Mar. 15, 2019	
	CL**	Matsuo et al BBRC 170 (1990) 963-967	

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	CM**	Matsuo "Molecular biological study to establish the treatment for Duchenne muscular dystrophy" Research Report of Grants-in-Aid for Scientific Research, Ministry of Education, March 1997 p.1, 5-13 (w/ English translation)	
	CN**	Nakajima et al J Neurol (1991) 238:6-8	
	CO**	Matsuo et al J Clin Invest. 1991;87(6):2127-2131	
	CP**	Narita et al J Clin Invest. 1993;91(5):1862-1867	
	CQ**	Suryono et al Proceedings of the Association of American Physicians 108 308-314 (1996)	
	CR**	JP Patent application No. 2000-125448, filed Apr. 26, 2000 (w/ English translation)	
	CS**	Alan et al Hum Genet (1990) 86:45-48	
	CT**	Matsuo "Establishment of treatment of Duchenne muscular dystrophy" Research Report of Grants-in-Aid for Scientific Research, Ministry of Education, March 2000 p.1, 5-11 (w/ English translation)	
	CU**	Marcusson et al., MOLECULAR BIOTECHNOLOGY, Volume 12, 1999, 1-11	
	CV**	Patentee's argument filed with JPO in JP Appl'n 2013-260728 on Apr.13, 2015	

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Examiner Name	Not Yet Assigned

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	CW**	Decision of Rejection by JPO in JP Appl'n 2011-098952 on Aug. 21, 2013	
	CX**	Patentee's argument filed with the JPO in Opposition of JP6126983 on Mar. 23, 2016 (w/ English translation)	
	CY**	David R Corey et al Genome Biology 2001 2(5) 1015.1-1015.3	
	CZ**	AU 2004903474 filed Jun. 28, 2004, priority document for PCT/AU05/000943	
	DA**	Experimental report submitted in EPO Opposition in EP 2206781, Aug. 25, 2016	
	DB**	Experimental report (D 8-1) submitted in EPO Opposition in EP 2206781, Sept. 29, 2017	
	DC**	Map of target region, submitted in EPO Opposition in EP 2206781, Feb. 22, 2017	
	DD**	Experimental report, submitted in EPO Opposition in EP 2206781, Feb. 22, 2017	
	DE**	Declaration by Fred Schnell, dated Sept. 28, 2017, submitted in EPO Opposition in EP 2206781, Sept. 29, 201756	
	DF**	Summerton et al Antisense&Nucleic acid drug development 7:187-195(1997)	

Examiner Signature		Date Considered	

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[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	22	of	26

Complete if Known	
Application Number	Not Yet Assigned
Filing Date	Concurrently Herewith
First Named Inventor	Naoki WATANABE
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	209658-0001-11-US-000003

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	DG**	Experimental report (D13), submitted in EPO Opposition in EP 2206781, Sept. 29, 2017	
	DH**	Declaration by Fred Schnell submitted in EP2206781 Opposition on Apr. 25, 2018	
	DI**	Amendment in response to Non-Final Office Action in US 15/705,172, filed Jan 5, 2018	
	DJ**	UNIVERSITY OF WESTERN AUSTRALIA MOTION 1 filed in U.S. Patent Interference No. 106,007 (RES), on Nov. 18, 2014	
	DK**	Prior et al., Human Genetics 92: 302-304 (1992)	
	DL**	Abstracts: 32nd European Muscle Conference, 'A link between fundamental research and therapeutic trials,' The Annual Meeting of the European Society for Muscle Research, Journal of Muscle Research and Cell	
	DM**	Wells et al., FEBS LETT. 2003 vol.552 145-149	
	DN**	Cagliani et al., Human Genetics June 2004 Vol.115 13-18	
	DO**	Bremmer-Bout et al., MOLECULAR THERAPY 2004 Vol.10 232-240	
	DP**	Abstracts of The Australasian Gene Therapy Society 4th Society Meeting, JOURNAL OF GENE MEDICINE AUG 2005 Vol.7, 1113-1143	

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Sheet	23	of	26

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Art Unit	N/A
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	DQ**	Editorial by Wilton et al., Neuromuscular Disorders 2005 Vol.15, 399-402	
	DR**	Specification of EP 12198465.2 filed Sept. 21, 2001	
	DS**	Applicant's letter mailed November 18, 2013 in EP 12198465.2	
	DT**	Observations by third parties submitted in EP3018211 Jun. 13, 2018	
	DU**	Communication from the Examining Division and Annex to the Communication issued in EP 3018211 on Nov. 9, 2018	
	DV**	Harding et al., Molecular Therapy, Vol. 15, No. 1, 157-166 (2007)	
	DW**	U.S. provisional application 61/108,416 filed Oct. 24, 2008, priority document of WO 2010/048586	
	DX**	Nishida et al., Nature Communications, Volume 2, Article number: 308 (2011)	
	DY**	Appellant University of Western Australia's Statement of Grounds for Appeal submitted in EP 2 206 781, dated April 27, 2018	
	DZ**	Nippon Shinyaku Co., Ltd.'s Reply to the Grounds of Appeal in EP 2 206 781, dated September 6, 2018	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet	24	of	26	Attorney Docket Number	209658-0001-11-US-000003

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Application Number	Not Yet Assigned
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Art Unit	N/A
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	EA**	Opposition filed by Nippon Shinyaku Co., Ltd. in EP 2 206 781, dated August 25, 2016	
	EB**	The University of Western Australia's reply to Opposition in EP 2 206 781, dated February 22, 2017	
	EC**	EPO's Opposition Division's Preliminary Opinion in EP 2 206 781 B1, dated March 30, 2017	
	ED**	EPO's Decision on Opposition in EP 2 206 781 B1, dated December 19, 2017	
	EE**	Final Office Action in U.S. patent application No. 16/243,926, dated May 15, 2019	
	EF**	Amendments in EP 3 404 100, dated May 13, 2019	
	EG**	Search opinion in EP 3 404 100, dated October 24, 2018	
	EH**	European search report dated July 26, 2019 issued in European patent application No. 19169673.1	
	EI**	Declaration by Judith C. van Deutekom executed October 10, 2019, submitted in Invalidation Trial of JP6126983, on October 10, 2019	
	EJ**	Vickers, Timothy A., et al., "Effects of RNA secondary structure on cellular antisense activity," Nucleic Acids Research, 2000, Vol. 28, No. 6, pp. 1340-1347	

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Sheet	25	of	26

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Art Unit	N/A
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	EK**	Johansson, Hans E., et al., "Target-specific arrest of mRNA translation by antisense 2'-O-alkyloligoribonucleotides," Nucleic Acids Research, 1994, Vol. 22, No. 22, pp. 4591-4598	
	EL**	Peyman, Anusch, et al., "Inhibition of Viral Growth by Antisense Oligonucleotides Directed against the IE110 and the UL30 mRNA of Herpes Simplex Virus Type-1," Biol. Chem. Hoppe-Seyler, March 1995, Vol. 376, pp. 195-198	
	EM**	Monia, Brett, P., et al., "Antitumor activity of a phosphorothioate antisense oligodeoxynucleotide targeted against C-*raf* kinase," Nature Medicine, June 1996, Volume 2, Number 6, pp. 668-675	
	EN**	Errington, Stephen J., et al., "Target selection for antisense oligonucleotide induced exon skipping in the dystrophin gene," J. Gene Med. 2003, Vol. 5, pp. 518-527	
	EO**	Morita, Koji, et al., "Synthesis and Properties of 2'-*O*,4'-*C*-Ethylene-Bridged Nucleic Acids (ENA) as Effective Antisense Oligonucleotides," Bioorganic & Medicinal Chemistry 2003, Vol. 11, pp. 2211-2226	
	EP**	Summerton, James E., "Morpholinos and PNAs compared," Letters in Peptide Science 2003, Vol. 10, pp. 215-236	
	EQ**	Ramsey, Joshua D., et al., "Cell-penetrating peptides transport therapeutics into cells," Pharmacology & Therapeutics 2015, Vol. 154, pp. 78-86	
	ER**	Declaration by Toshihiro Ueda submitted in Opposition Proceeding of EP 3018211, dated September 29, 2020, submitted October 27, 2020	
	ES**	Opposition filed by Sarepta Therapeutics, Inc. in EP 3018211, dated May 5, 2020	
	ET**	Opposition filed by James Poole Limited in EP 3018211, dated May 7, 2020	

Examiner Signature		Date Considered	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet	26	of	26	Attorney Docket Number	209658-0001-11-US-000003

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Application Number	Not Yet Assigned
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	EU**	Nippon Shinyaku Co.'s reply to Oppostion in EP 3018211, dated October 27, 2020	
	EV**	LINDA J. POPPLEWELL et al., "Design of Phosphorodiamidate Morpholino Oligomers (PMOs) for the Induction of Exon Skipping of the Human DMD Gene," Mol. Ther., Vol. 17, no. 3, March 2009, pp. 554-561, Supplemental Table S1.	
	EW**	LINDA J. POPPLEWELL et al., "Design of Phosphorodiamidate Morpholino Oligomers (PMOs) for the Induction of Exon Skipping of the Human DMD Gene," Mol. Ther., Vol. 17, no. 3, March 2009, pp. 554-561, partial enlargement of Supplemental Table S1.	
	EX**	Sarepta R&D Day 2018- Presentation (June 19, 2018) (available at https://investorrelations.sarepta.com/static-files/37f91de4-480e-4429-b453-18ba373fe599)	

Examiner Signature		Date Considered	

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Docket No.: 209658-0001-11-US-000003
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Naoki WATANABE et al.

Confirmation No.: Unassigned

Continuation Application of
Prior Application No.: 17/126,366
Prior Application Filed: December 18, 2020

Group Art United: Not Yet Known

Continuation Filed: Concurrently Herewith

Examiner: Not Yet Known

For: ANTISENSE NUCLEIC ACIDS

INFORMATION DISCLOSURE STATEMENT (IDS)

MS NEW
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 C.F.R. § 1.56, 1.97 and 1.98, the attention of the Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

This Information Disclosure Statement is being filed concurrently with the instant continuation application. Accordingly, no fee is due for filing this paper.

The listed documents have been submitted in the prior parent application No. 17/126,366, having a filing date of December 18, 2020, upon which Applicants rely for the benefits provided in 35 U.S.C. § 120. Accordingly, pursuant to 37 C.F.R. § 1.98(d), a copy of each reference is not provided. Applicants also call the Examiner's attention to any U.S., International, and/or

Foreign search reports, Office Actions, and the like that may have been filed by Applicants in the prior patent application No. 17/126,366 for the Examiner's consideration.

Applicants respectfully request that the Examiner consider the listed documents and evidence that consideration by making appropriate notations on the attached form PTO/SB/08.

This submission does not represent that a search has been made or that no better art exists and does not constitute an admission that any of the listed documents is material or constitutes "Prior Art." If it should be determined that any of the listed documents do not constitute "Prior Art" under United States law, Applicants reserve the right to present to the Office the relevant facts and law regarding the appropriate status of such documents.

Applicants further reserve the right to take appropriate action to establish the patentability of the disclosed invention over the listed documents, should one or more of the documents be applied against the claims of the present application.

Applicants believe no fee is due with this response. However, if a fee is due, the Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 50-0573, under Order No. 209658-0001-11-US-000003 from which the undersigned is authorized to draw.

Dated: February 12, 2021

Respectfully submitted,

FAEGRE DRINKER BIDDLE & REATH LLP

/Zhengyu Feng/
Zhengyu Feng, Ph.D. – Reg. No. 66,816

CUSTOMER NO. 055694

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